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(54) Carboxyalkyl peptide derivatives.

(57) This invention encompasses novel carboxyalkyl peptide derivatives which are collagenase inhibitors

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BACKGROUND OF THE INVENTION

This invention relates to novel compounds having pharmacological activity, to the production thereof, to compositions containing them, and to their use in pharmacy.

A number of compounds have been described which are competitive reversible inhibitors of zinc-containing metalloproteinase enzymes. Such competitive reversible inhibitors are for example those which are inhibitors for the angiotensin converting enzymes (ACE). The utility of such an inhibitor is that it acts to block conversion of the decapeptide angiotensin I to antiotensin II, this last-mentioned compound being a potent pressor substance. ACE inhibitors are therefore potentially of use in the treatment of hypertension. Compounds of this type are for example described in European Patent Application A-0012401. Related inhibitors of the enzyme enkephalinase are described in EPA 0054862.

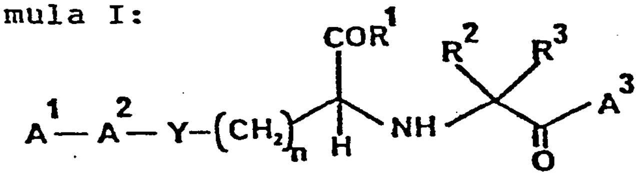
We have found a group of compounds which act as inhibitors of mammalian collagenase [EC 3.4.24.7] which initiates collagen breakdown. There is now compelling evidence [see for example Arthritis and Rheumatism, 20, 1231, (19770] implicating the involvement of the zinc metalloproteinase, collagenase, as one of the key enzymes in the degradation of articular cartilage and bone in rheumatoid arthritis. Collagen is ont of the major components of the protein matrix of cartilage and bone. Potent inhibitors of collagenase are useful in the treatment of rheumatoid arthritis and associated diseases in which collagenolytic activity is a contributing factor. These diseases include corneal ulceration, periodontal disease, tumour invasion and dystrophic epidermolysis bullosa.

These compounds have substantially no ACE-inhibiting-activity. ACE is a carboxydipeptidase - it cleaves a peptide substrate two residues from the C-terminus. Consequently the C-terminal carboxylic acid is a prime recognition site for both substrates and inhibitors;

removal of this ionic binding group drastically reduces inhibitory potency. Collagenase, on the other hand, is an endopeptidase and, as such, has no prerequisite for this binding interaction. Additionally the structure of collagen differs essentially from angiotensin-I, which as noted above is a decapeptide and is cleaved at a phenylalanine-histidine bond to give an octapeptide (angiotensin-II) and a dipeptide (histidylleucine). Collagen is much more complex, in being a triple helix, each strand of the helix containing of the order of 1,000 amino acid residues, the sequence of amino acids around the site cleaved by collagenase being completely different from that around the cleavage site of Angiotensin I. Collagenase cleaves this triple helix at a single locus on each chain approximately two-thirds of the way along the chain from the N-terminus. The amide bond which is cleaved by collagenase is either a glycine-leucine or a glycine-isoleucine bond.

BRIEF DESCRIPTION OF THE INVENTION

The present invention provides compounds of the general formula I:



I

and pharmaceutically acceptable salts thereof in which

n = 1-4

R^1 represents hydroxy, alkoxy, aralkoxy or hydroxy-amino;

R^2 represents hydrogen or alkyl;

R^3 represents hydrogen,

alkyl,

substituted alkyl wherein the substituent may be one or more of the groups selected from hydroxy, alkoxy, aryloxy, aralkoxy, mercapto, alkylthio, arylthio, alkylsulphinyl (e.g. $SOCH_3$), alkylsulphonyl (e.g. SO_2CH_3), carboxy, carboxamido (e.g. $CONH_2$), carboxyalkyl (e.g. CO_2CH_3), carboxyaralkyl (e.g. CO_2CH_2Ph), aralkoxycarbonylamino (e.g. $NHCOOCH_2Ph$), amino, dialkylamino, acylamino (e.g. $NHCOCH_3$), aroylamino (e.g. NHCOPh) and trihalomethyl (e.g. CF_3),

aralkyl,

substituted aralkyl wherein the substituent on the aryl moiety may be one or more groups selected from halogen (e.g. fluorine, chlorine, bromine, iodine), alkyl, hydroxy, alkoxy, aralkoxy, amino, aminomethyl (CH_2NH_2), cyano, alkylamino, dialkylamino, carboxy, sulphonamido, alkylthio, nitro and phenyl,

or heteroaralkyl;

Y represents NR^4 wherein R^4 represents H or alkyl; or for certain values of A^1, A^2 may alternatively be a direct chemical bond.

When Y represents NR^4,

A^1 represents a group of formula R^5 wherein

R^5 may be hydrogen,

alkyl,

aralkyl,

aryl,

substituted aryl wherein the substituent may be one or more groups selected from halogen alkyl, hydroxy, alkoxy, aralkoxy, aralkoxyamino, aminomethyl, cyano, acylamino, dialkylamino, carboxy, sulphonamido, alkylthio, nitro and phenyl,

acyl (e.g. CH_3CO),

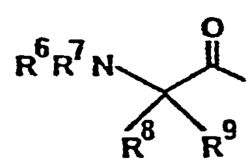
aroyl (e.g. PhCO),

aralkylacyl (e.g. $PhCH_2CO$),

alkoxycarbonyl (e.g. $(CH_3)_3OCO$),

or aralkoxycarbonyl (e.g. $PhCH_2OCO$);

A^1 may also represent a group of the formula:



wherein R^6 represents a group having the meanings defined above for R^5;

R^7 and R^8 which may be the same or different represent hydrogen, alkyl or aralkyl; or

R^7 and R^8 may together represent an alkylene chain of 2-4 carbon atoms so to form with the adjacent nitrogen atom a nitrogen-containing ring having 4-6 atoms;

R^9 represents hydrogen,

alkyl,

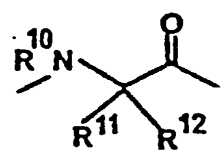
substituted alkyl wherein the substituent is exactly as defined for this moiety above,

aralkyl,

substituted aralkyl wherein the substituent is exactly as defined for this moiety above,

or heteroaralkyl;

A^2 represents a group of the formula



wherein

R^{10} and R^{11} which may be the same or different represent groups having the meanings given above for R^7 or together represent an alkylene chain of 2-4 carbon atoms so as to form with the adjacent nitrogen a nitrogen-containing ring having 4 to 6 atoms;

R^{12} represents a group having the meanings given above for R^9.

Additionally, A^1 and A^2 taken together may represent

hydrogen,

alkyl,

aralkyl,

heteroaralkyl,

alkylsulphonyl,

arylsulphonyl,

aralkylsulphonyl,

or a group $R^{13}CO$ wherein R^{13} represents

hydrogen,

alkyl,

alkoxy,

aryl,

aralkyl,

aralkoxy,

substituted aryl (as defined in R^3),

substituted aralkyl (as defined in R^3) and

substituted aralkoxy wherein the substituent on the aromatic moiety of the aralkoxy is as defined for aralkyl

phenethenyl (PhCH=CH-),

phenethynyl (PhC≡C-),

alkylamino,

arylamino,

aralkylamino,

or dialkylamino;

In a further aspect of this invention, Y may also represent a direct chemical bond. In this instance, A^1 and A^2 taken together represent

hydrogen,

alkyl,

aryl,

alkoxy,

aralkoxy,

substituted aryl (as in R^3) and substituted aralkoxy (as in R^3) wherein the substituent on the aromatic moiety of the aralkoxy is as defined for aralkyl,

hydroxy,

mercapto,

alkylthio,

arylthio,

aralkylthio,

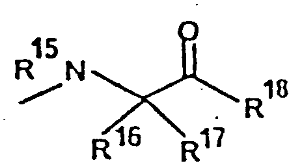
carboxy,

or carboxyalkyl;

A^3 represents a group of the formula

R^{14}

or



wherein

R^{14} represents amino,

alkylamino,

dialkylamino,

hydroxyamino,

or aralkylamino,

and R^{15}, R^{16} and R^{17} which may be the same or different represent groups having the meaning given above for R^{10}, R^{11} and R^{12} respectively and

R^{18} represents amino,

alkylamino,

dialkylamino,

substituted alkylamino wherein the substituent is amino, hydroxy, alkoxy, carboxy, carboxamido, carboxyalkyl, alkylthio, alkylsulphinyl or alkylsulphonyl,

hydroxyamino,

alkoxyamino,

aralkylamino,

alkoxy,

aralkoxy,

or alkylaminoalkoxy.

all with the exception that when A^3 is alkylamino one of R^2 and R^3 is not hydrogen and the other alkyl or hydroxyalkyl.

DETAILED DESCRIPTION OF THE INVENTION

The term alkyl as used herein to designate a group or a part thereof includes reference to both straight and branched alkyl groups and to cycloalkyl groups which may contain from 1 to 10, preferably 1 to 6, carbon atoms in the case of straight or branched chain non-cyclic alkyl groups (for example methyl, ethyl, propyl,isopropyl) and from 3 to 10, preferably 3 to 7 in the case of cyclic alkyl groups (for example cyclopentyl, norbornyl).

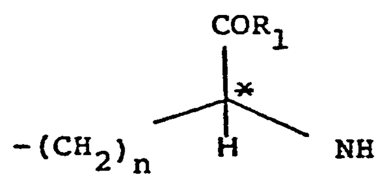
By the term aryl, is meant phenyl or naphthyl.

The terms aralkyl and aralkoxy include in particular those groups containing 1 to 4 carbon atoms in the alkyl portion, and those groups in which aryl has the meaning just given.

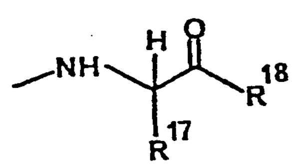
By the term heteroaralkyl we mean in particular groups containing 1 to 4 carbon atoms in the alkyl moiety. The term heteroaryl includes for example, pyridyl, thienyl, furyl, indolyl, imidazolyl and thiazolyl.

Typical pharmaceutically acceptable addition salts are those derived from mineral and organic acids such as hydrochloric, hydrobromic, hydroiodic, p-toluene sulphonic, sulphuric, perchloric, acetic, benzoic, trifluoroacetic and the like.

There are several chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. These centres may be racemised or in any optically active form. We have found surprisingly that those compounds in which the chiral centre indicated below by an asterisk in the group shown is in the R form are preferred.



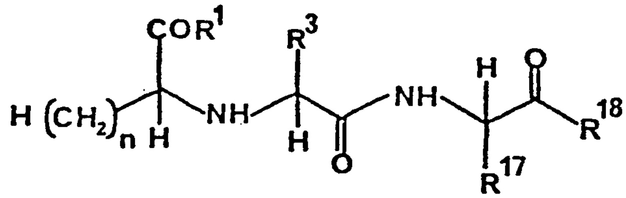
Certain groups of compounds according to the invention are preferred, these including the following. A group of preferred compounds are those in which the group A^3 has the following meaning



in which R^{17} represents substituted alkyl (wherein the substituent is alkoxy, aralkoxy, alkoxycarbonylamino, aralkoxycarbonylamino, carboxyalkyl or carboxyaralkyl); or substituted aralkyl (wherein the aryl substituent is one or more groups selected from alkyl, alkoxy, alkyl thio or aralkoxy). In this preferred group of compounds, R^3 should have the meanings described hereinbefore but excluding aralkyl or heteroalkyl.

Within this definition of A^3, there is a preferred subclass of compounds in which $A^1 + A^2$ taken together represent H, Y is a direct chemical bond, R^2 represents H, and R^3 represents alkyl or substituted alkyl where the substituent(s) is one or more trifluoromethyl groups.

Therefore this first sub-class of preferred compounds may be defined by the formula



wherein R^3 and R^{17} are as defined above. A most preferred set of compounds within this group are those in which R^{17} is benzyloxymethyl ($PhCH_2OCH_2$-), 1-benzyloxyethyl ($PhCH_2OCH(CH_3)$-), 4-benzyloxyphenylmethyl (4-$PhCH_2OC_6H_4CH_2$-) or 4-methoxyphenylmethyl (4-$CH_3OC_6H_4CH_2$-).

In a second preferred sub-class of compounds within the preferred definition of A^3, Y represents NR^4, and $A^1 + A^2$ represent a group $R^{13}CO$ wherein R^{13} represents alkyl,

aryl,

aralkyl,

aralkoxy,

substituted aryl, substituted aralkyl and substituted aralkoxy wherein the substituent on the aromatic moiety is exactly as defined hereinbefore,

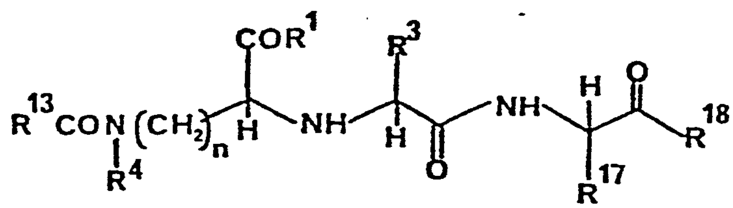
alkylamino,

arylamino,

aralkylamino

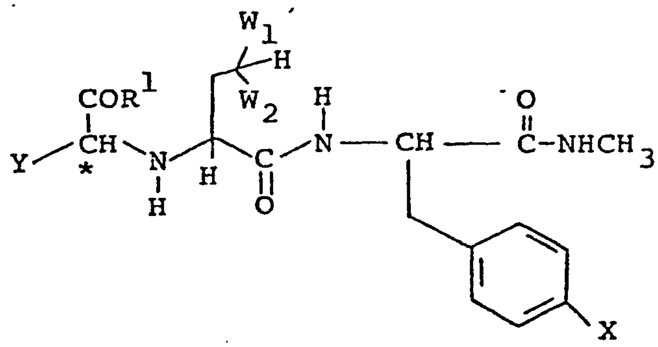
or dialkylamino.

Therefore this second sub-class of preferred compounds may be defined by the formula

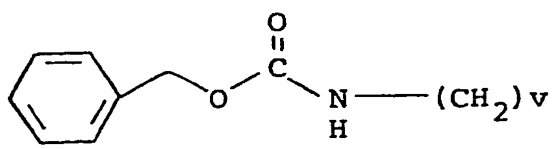


Particularly preferred examples are those in which R^4 is H and R^3, R^{17} and R^{18} are as defined for the first preferred sub-class of compounds. A most preferred series of compounds within this sub-class is where n is 2, R^{13} is benzyloxy ($PhCH_2O$), substituted benzyloxy (where the aromatic substituent is selected from 4-chloro, 2-chloro, 4-methyl, 4-nitro or 4-amino), benzylamino ($PhCH_2NH$), phenyl or substituted phenyl (where the aromatic substituent is selected from 4-chloro, 2-chloro, 4-methyl, 4-nitro or 4-amino).

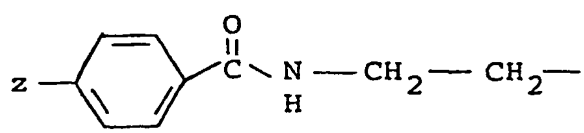
A further preferred embodiment of the invention is a compound of the formula



and the pharmaceutically acceptable acid addition salts thereof wherein x represents hydrogen, alkoxy or benzyloxy; y represents a radical selected from alkyl, alkylthioalkyl,



wherein v is 2 or 3,



wherein z represents hydrogen or nitro; W_1 and W_2 represent methyl or trifluoromethyl; and R^1 represents hydroxy or alkoxy and the stereochemistry of the carbon marked by the asterisk is R.

Specific compounds according to the invention are those, the preparation of which is described in the Examples.

The compounds according to the invention exhibit inhibitory action against collagenase. This was determined following the procedure of Cawston and Barrett, <u>Anal. Biochem.</u>, <u>99</u>, 340-345 (1979) whereby the 1mM of the inhibitor being tested or dilutions thereof are incubated at 37°C for 16 hours with native collagen and collagenase (buffered with Tris HCl-$CaCl_2$; pH 7.6). The collagen is acetyl ^{14}C collagen. The samples are centrifuged to sediment undigested collagen and an aliquot of the radiactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM inhibitor, or a dilution thereof, is compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the collagenase. Table II illustrates the activity of compounds of this invention.

For use in treatment of rheumatoid arthritis the compounds of this invention can be administered by any convenient route preferably in the form of a pharmaceutical composition adapted to such route and in a dose effective for the intended treatment. In the treatment of arthritis administration may conveniently be by the oral route or by injection intraarticularly into the affected joint. The daily dosage for a 70 kilogram mammal will be in the range of 10 milligrams to 1 gram.

The compounds of this invention can be formulated in compositions such as tablets, capsules or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. About 10 to 500 mg of a compound according to the invention is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavour, etc., in a unit dosage from as called for by accepted pharmaceutical practice. (See for example, Remington's Pharmaceutical Science Mach Publishing Co., Easton, Penn. 1965). The amount of active substance in these compositions or preparations is such that a suitable dosage in the range indicated is obtained.

The compounds according to the invention may be made by methods which are generally known in peptide chemistry for analogous compounds. In particular it is to be understood that reactive groups not involved in a particular reaction (e.g. amino, carboxy, hydroxy etc.,) may be protected by methods standard in peptide chemistry prior to reactions of other groups and subsequently deprotected.

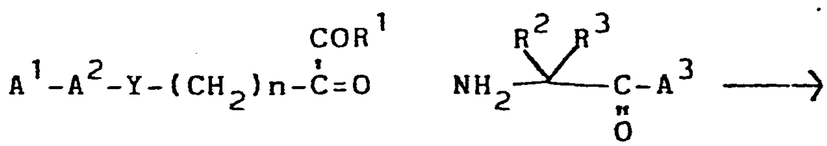
The intermediates of use in the production of the end-products are either known compounds or can be made by known methods, as described in the Examples.

The following description of the preparative methods

indicates generally the routes which may be used for the production of the compounds according to the invention.

Process 1, Route A

This process involves reductive amination



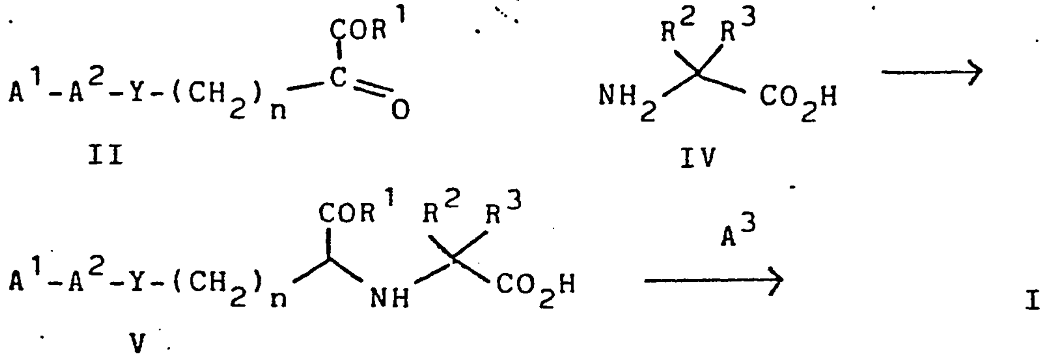
I

II III

A keto acid (or derivative) of formula II is condensed with a peptide of formula III . This condensation is conveniently carried out in a suitable solvent (e.g.aqueous tetrahydrofuran,methanol) at a pH between 6 and 7 in the presence of sodium cyanoborohydride which effects reduction to give the desired compound of formula I. Alternatively, II and III may be reacted in the solvent medium to form a Schiff's Base as an intermediate and this may then be reduced catalytically to yield the desired compound of formula I for example by hydrogenation in the presence of Raney Nickel or palladium on charcoal.

As an alternative to Process , Route A , the compound of formula II can be condensed with an amino acid of formula IV below (or protected derivative thereof) under the same conditions as given in Process 1 to yield an intermediate of formula V. This intermediate is then

subsequently coupled with an amino acid or peptide derivative of the formula A^3 to give the compound of formula I.

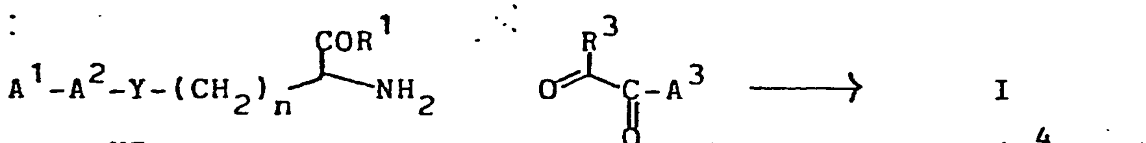


The known processes for peptide bond formation referred to above and also in the following processes encompass reactive group protection during the coupling reaction, e.g., for amines employing N-t-butyloxycarbonyl or N-benzyloxycarbonyl followed by their removal. Condensing agents are typically those useful in peptide chemistry such as dicyclohexylcarbodiimide, water soluble carbodiimide [N-ethyl-N^1-(3-dimethylaminopropyl)-carbodiimide], diphenyl phosphoryl azide or V may be activated via the intermediary of active esters such as those derived from 1-hydroxybenzotriazole, 4-nitro phenol, 4-picolyl alcohol.

<u>Process 1 Route B</u> (where R^4 = H)

In an alternative reductive amination process as shown below, the starting materials providing the groups A^1-A^2 on the one hand and the group A^3 on the other are reversed. Otherwise the process is the same as

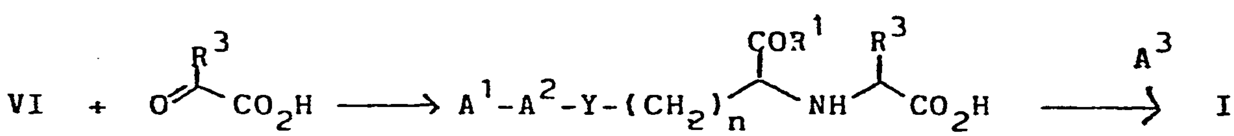
Process 1 Route A . This process is applicable to the production of compounds in which $R^4 = H$.



VI VII ($R^4 = H$)

The amino acid (or derivative) VI is condensed with the ketone (VII) under the conditions given in Route A .

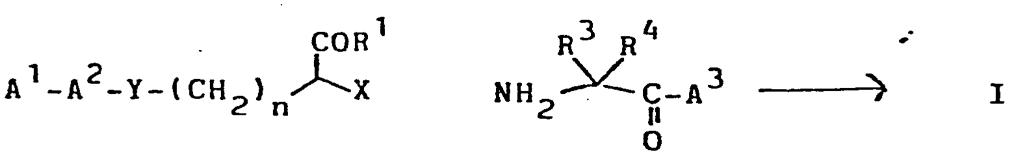
As an alternative to Process 1, Route B the synthesis can be performed in a step wise manner by condensing VI with the keto acid (or derivative) VIII to yield the intermediate IX. By known processes (summarised above), IX can then be condensed with an amino acid or peptide derivative A^3 to give I.



VIII IX (R^4=H)

<u>Process 2 Route A</u>

This process is essentially an alkylation.

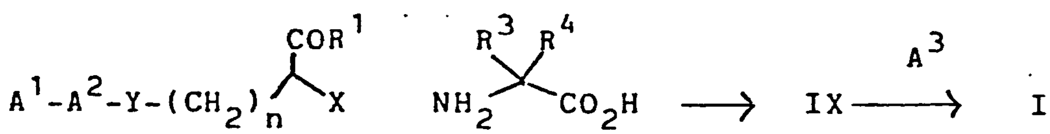


X III

In this process the peptide III is alkylated with the

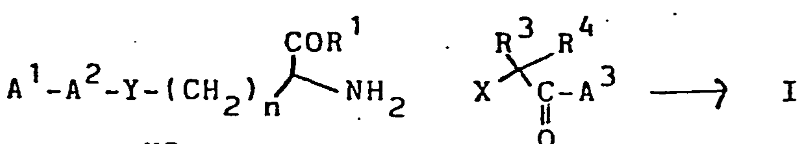
appropriate α-haloacid (or derivative) X or α-sulphonyloxy acid in a suitable solvent (e.g. CH_2Cl_2, CH_3CN, etc.) in the presence of a base (e.g. triethylamine).

As an alternative to this process, the synthesis can be performed in a stepwise fashion firstly to produce an intermediate IX which is then condensed by standard processes above with a peptide derivative A^3 to give the compound of formula I, as described above for the alternative for Process 1, Route A .



Process 2 Route B

In an alternative alkylation shown below the starting materials providing the groups A^1-A^2- on the one hand and A^3 on the other are reversed. Otherwise the method is the same as Process 2, Route A .

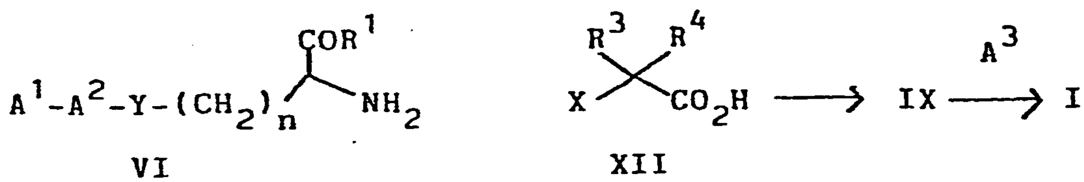


VI XI

The amino acid (or derivative) VI is alkylated with the α-haloacetyl or α-sulphonyloxyacetyl peptide derivative XI under the conditions described in Route A .

As an alternative to Process 2, Route B the synthesis can be performed in a stepwise fashion by

condensing an amino acid (or derivative) VI with a substituted α-haloacetic acid or α-sulphonyloxy acetic acid (XII) to yield the intermediate IX which by standard processes is condensed with a peptide derivative A^3 to give the compound of formula I



It should be noted that when A^1 and/or A^2 represent amino acid residues, that these residues may be introduced by standard coupling procedures at any convenient stage of the synthesis.

The starting materials which are required for the above processes are either known in the literature, or can be made by standard processes from known starting materials, or are described in the Examples.

When R^1 in I represents hydroxy, these compounds may be derived from those described above (wherein R^1 = alkoxy or aralkoxy) by hydrolysis in a suitable solvent (such as aqueous methanol) containing a base such as sodium or lithium hydroxide. Alternatively, when R^1 = aralkoxy (such as $PhCH_2O$), this group may be removed by hydrogenolysis.

As mentioned above there are various potentially

asymmetric centres in the amide derivatives of this invention. In particular the carbon atom which bears the groups $(CH_2)_n$, COR^1 and NH is asymmetric as is that which bears the groups NH, COA^3, R^2 and R^3 (when R^2 and R^3 are not simultaneously hydrogen). The above synthesis can utilize racemates, enantiomers or diastereoisomers as starting materials, the products can therefore exist in racemic or optically active forms. The invention therefore encompasses the racemic form as well as any other optically active forms. As noted above, however, and in contrast to inhibitors of other zinc metalloproteinases (such as angiotensin converting enzyme), the preferred isomer has R-stereochemistry at the carbon atom bearing the groups $(CH_2)_n$, COR^1 and NH whilst having the stereochemistry of the natural amino acids at the other asymmetric centres.

The compounds according to the invention include pharmaceutically acceptable salts of the compounds of formula I. Such salts may include acid addition salts as well as amine salts, etc., and the processes described above for the production of the compounds may include as a final step the conversion of the compound I into such a salt, or the compound may be isolated as such salt.

It is understood that the compounds which bind most effectively to collagenase have R^1 equal to either hydroxy or hydroxyamino. When R^1 is alkoxy or aralkoxy, these compounds function as orally active prodrugs of the parent carboxylic acids; once absorbed these esters are rapidly hydrolysed by non specific plasma esterases to yield the active species.

In order that the invention may be more fully

understood the following Examples are given by way of illustration and should not limit the invention in spirit or scope.

Example 1

N-(1-Methoxycarbonylethyl)-L-leucyl-L-valine N-Hexylamide

N-(Tertiarybutyloxycarbonyl) -L-leucyl-L-valine N-Hexylamide (2g) was treated with trifluoroacetic acid (20ml) at room temperature for forty-five minutes. The excess trifluoroacetic acid was removed *in vacuo* and the residue dissolved in methanol (20ml). The solution was adjusted to pH7 with triethylamine. Dried 3A molecular sieve (10g), sodium cyanoborohydride(0.75g) and methyl pyruvate (1.5g) were added and the reaction mixture stirred at room temperature for 2 days. The reaction mixture was then filtered and the filtrate concentrated *in vacuo* to a gum. The residue was taken up in dichloromethane and the organic phase washed in turn with saturated sodium hydrogen carbonate solution and then 1M citric acid solution and dried over sodium sulphate. The material isolated after evaporation of the dichloromethane was chromatrographed on silica, developed in a gradient of 20% ethyl acetate in hexane to 60% ethyl acetate in hexane. Elution with 40% ethyl acetate hexane afforded N[1-(S)-methoxycarbonylethyl]-L-leucyl-L-valine N-hexylamide (0.4g), which crystallised from methanol/water as

needles m.p. 70-71°C; $[\alpha]^{20}_{D}$ = -31.4° (c = 0.2, MeOH); (Found: C,63.0; H,10.2; N,10.5. $C_{21}H_{41}N_3O_4$ requires C,63.1; H,10.3; N,10.5%); ν_{max} (Nujol): 3400, 1740 and 1610 cm^{-1}; (a) δ ($CDCl_3$) 0.9 (15H, m, 2xCH$(CH_3)_2$ and $CH_2C\underline{H}_3$); 1.3(3H, d, J=6Hz, $C\underline{H}_3$CH); 1.2-2.4 (12H, m, 2xC$\underline{H}$$(CH_3)_2$, CHC$\underline{H}_2$CH and $(CH_2)_4$); 3.0-3.4 (5 H, m, 3x C$\underline{H}$ and C$\underline{H}_2$NH); 3.7 (3H,s; CH_3-O), 4.3(1H, t, J= 8Hz,NH); 6.94 (1H,m,NH) and 7.85 (1H,d,NH).

Elution with 50% ethyl acetate hexane afforded <u>N[1-(R)-methoxycarbonylethyl]-L-leucyl-L-valine N-hexylamide</u>, (0.5g) which crystallised from methanol/ water as needles m.p. 98-101°C; $[\alpha]^{20}_{D}$ = -43° (C=0.2, MeOH); (Found: C,62.7; H,10.2; N,10.5. $C_{21}H_{41}N_3O_4$ requires C,63.1; H,10.3; N,10.5%); ν_{max} (Nujol) 3250, 3060, 1730 cm^{-1}; δ($CDCl_3$) 0.9 (15H,m, 2xCH$(C\underline{H}_3)_2$ and $CH_2\underline{CH_3}$); 1.3 (3H,d;J=6Hz, $C\underline{H}_3$ CH); 1.2-2.4 (12H,m, 2xC$\underline{H}$$(CH_3)_2$, CHC$\underline{H}_2$CH and $(C\underline{H}_2)_4$); 3.0-3.3 (4H,m, 2xNHC$\underline{H}$CO,CH_2); 3.44(1H,q,J=7Hz,val α-C$\underline{H}$); 3.7(3H,s CH_3-O); 4.28 (1H, q. J=7Hz,NH); 7.16 (1H,m,NH); and 7.92 (1H,d, J=8Hz,NH).

The N-(t-butyloxycarbonyl)-L-leucyl-L-valine N-hexylamide used as a starting material was prepared as follows:

N-Tertiarybutyloxycarbonyl-L-valine N-hexylamide (15g) in dichloromethane (30ml) was treated with tri-

fluoroacetic acid (30ml) at room temperature for 45 minutes. The excess trifluoroacetic acid was removed *in vacuo* and the residue redissolved in dichloromethane. The solution was adjusted to pH7 with triethylamine, N-tertiarybutyloxycarbonyl-L-leucine (13g), 1-hydroxy-benzotriazole (7g) and DCC (10g) were added and the reaction mixture stirred at room temperature overnight. The reaction mixture was filtered and the organic phase washed with aqueous sodium hydrogen carbonate, 1M citric acid and then water, dried over sodium sulphate and concentrated *in vacuo* to a gum. The gum was chromatographed on silica developed in a gradient of 20% ethyl acetate to 50% ethyl acetate in petrol to afford N-tertiarybutyloxycarbonyl-L-leucyl-L-valine N-hexylamide (19g) which crystallised from ether hexane as needles; m.p. 115-116°C; (Found: C,63.2; H,10.3; N,10.1. $C_{22}H_{43}N_3O_4 \cdot 1/4H_2O$ requires C,63.2; H,10.5; N 10.1%); ν_{max} (nujol) 3300, 3080, 1680, 1630 and 1520 cm^{-1}; δ ($CDCl_3$) 0.9 (15H,m, $2xCH(C\underline{H}_3)_2$ and $CH_2C\underline{H}_3$); 1.1-2.3 (12H,m, $(C\underline{H}_2)_4$, $C\underline{H}_2C\underline{H}(CH_3)_2$, $CHC\underline{H}(CH_3)_2$); 1.45 (9H,s,$C(CH_3)_3$); 3.25 (2H,m,$NHC\underline{H}_2$); 4.12 (1H,m, α-CH from leucyl residue); 4.2 (1H,t, J=5Hz., α-$C\underline{H}$ from valyl residue); 5.07 (1H,m,NH); 6.55 (1H,m,NH) and 6.80 (1H,d,J = 10 Hz, NH).

The N-t-butyloxycarbonyl-L-valine N-hexylamide required as a starting material in the preparation

above was synthesised as follows:

Tertiarybutyloxycarbonyl-L-valine (25g) in dichloromethane (200ml) was treated with 1-hydroxybenzotriazole (15.5g) hexylamine (11.6g) and DCC (26g) at room temperature for 2 days. The solution was filtered and the organic phase washed with aqueous sodium hydrogen carbonate, aqueous citric acid (1M) and water, dried over sodium sulphate and concentrated *in vacuo* to afford tertiary butyloxycarbonyl-L-valine N-hexylamide (28g) which crystallised from methanol-water as needles; m.p. 74-76°C; (Found: C,63.8; H,10.6; N,9.4. $C_{16}H_{32}N_2O_3$ requires C,64.0; H,10.7; N,9.32%); ν_{max} (Nujol): 3280 and 1630 cm^{-1}; δ($CDCl_3$) 0.8-1 (9H,m, 3 x C$\underline{H}_3$); 1.3 (8H,m,(C$\underline{H}_2$)$_4$); 1.45 (9H,s, (C$\underline{H}_3$)$_3$C); 2.1 (1H,m,C$\underline{H}$(CH_3)$_2$); 3.3 (2H,m, NHC$\underline{H}_2$); 3.9 (1H,dd, J=8Hz and 5Hz.,α-C$\underline{H}$); 5.2 (1H,d, J = 8Hz, CONH) and 6.26 (1H,m,NH).

Example 2

N-[1-(R)-Methoxycarbonylethyl]-L-leucyl-O-benzyl-L-tyrosine N-Methylamide

N-Boc-O-benzyl-L-tyrosine methylamide (3g, 7.7mM) was dissolved in 1:1 TFA/CH_2Cl_2 (100ml). After 15 min. the solvent was removed *in vacuo* and the residue taken up in H_2O (100 ml), neutralised with $NaHCO_3$ and extracted into CH_2Cl_2 (3 x 100 ml). The organic extract

was dried and evaporated *in vacuo* to yield a white solid (2.2g). This material in CH_2Cl_2 (50 ml) and DMF (5 ml) was treated at 0° with N-[1-(R)-methoxycarbonylethyl]-L-leucine (1.3g, 6mM), 1-hydroxybenzotriazole (960 mg, 6.4mM) and dicyclohexylcarbodiimide (1.3g, 6.5mM) and the mixture allowed to warm to room temperature over 2h. After a further 12h the reaction mixture was filtered, washed with sat. $NaHCO_3$ and then brine, dried and then evaporated *in vacuo* to yield a solid, 2.5g (68%). Recrystallisation from CH_2Cl_2/hexane gave the title compound; m.p.65-68°; $[\alpha]_D^{20}$ = -3.3° (C=0.2, MeOH); (Found: C, 66.72; H, 7.61; N, 8.72. $C_{27}H_{37}N_3O_5$ requires C, 67.02; H, 7.71; N, 8.69%); ν_{max} (nujol) 3280 br, 1735, 1635 and 1510 cm^{-1}; $\delta(CDCl_3)$ 0.87 and 0.9 (each 3H, each d, J=4Hz. and 2.5 Hz, $(C\underline{H}_3)_2CH$); 1.1(1H,m, $(CH_3)_2CH_2C\underline{H}$); 1.3 (3H,d,J=8.5 Hz., $C\underline{H}_3CH$); 1.45 (2H,m, $(CH_3)_2C\underline{H}_2CH$); 1.58 br (1H,s, CHN$\underline{H}$CH, exch); 2.77 (3H,d,J=6Hz, $NHC\underline{H}_3$); 3.0 (1H,dd,J=12 and 8Hz, $C\underline{H}_2C_6H_4$); 3.07 (1H,m, $(CH_3)_2CH_2C\underline{H}$); 3.18 (1H,dd,J=12 and 6Hz, $CHC\underline{H}_2C_6H_4$); 3.38 (1H,q,J=8.5Hz, $CH_3C\underline{H}$); 3.68 (3H,s,OCH_3), 4.62 (1H,q,J=7Hz, $NHC\underline{H}(CH_2C_6H_4)CO$); 5.02 (2H,s,$OC\underline{H}_2C_6H_5$); 6.71 br (1H,q,J *ca* 5Hz, exch. $N\underline{H}CH_3$); 6.89 and 7.12 (each 2H, each d, each J=8Hz, C_6H_4); 7.4 (5H,m,C_6H_5) and 7.75 (1H,d, J=9Hz, exch, CON$\underline{H}$CHCO); m/e 484 (100%, $[M + 1]^+$), 381 (27) and 172 (28).

The syntheses for the two starting materials required in the preparation above are described in the following

paragraphs.

(a) N-t-Butyloxycarbonyl-O-benzyl-L-tyrosine N-Methylamide

N-t-Butyloxycarbonyl-O-benzyl-L-tyrosine (7.4g, 20mM), 1-hydroxybenzotriazole (3g, 20mM), methylamine hydrochloride (1.3g, 20mM) and N-methyl morpholine were dissolved in CH_2Cl_2 (200 ml) and cooled to 0°C. Dicyclohexylcarbodiimide (4.2g, 20mM) was added and the reaction allowed to warm to room temperature over 4h. After a further 12h the reaction mixture was filtered; the filtrate was washed with sat $NaHCO_3$, 3N citric acid and brine, dried and evaporated in vacuo to give the required N-methylamide which was recrystallised from CH_2Cl_2 and hexane (4.5g, 58%); m.p. 165-172°; $[\alpha]_D^{20}$ = +15.2° (C=0.2, MeOH); (Found: C, 68.85; H, 7.43; N, 7.39. $C_{22}H_{28}N_2O_4$ requires C, 68.73; H, 7.34; N,7.29%); ν_{max}(nujol) 3330, 1685, 1672, 1655 and 1520 cm^{-1}; δ ($CDCl_3$) 1.4 (9H,s, $(C\underline{H}_3)_3C$); 2.91 (3H,d, J=5Hz, $NHC\underline{H}_3$); 3.0 (2H,m,$C\underline{H}_2C_6H_4$); 4.26 (1H,q, J=7.5Hz, $NHC\underline{H}(CH_2)CO$); 5.04 (2H,s, $OC\underline{H}_2C_6H_5$); 5.08 br (1H,s, exch, NH); 5.84 br (1H,s,exch); 6.91 and 7.09 (each 2H, each d, each J=8Hz., C_6H_4) and 7.4 (5H,m,C_6H_5); m/e 385 (68%, $[M+1]^+$), 329 (100), 285 (66) and 267 (58).

(b) N-[1-(R)-Methoxycarbonylethyl]-L-leucine

This was prepared in two steps from L-leucine benzyl ester as illustrated below:

L-Leucine benzyl ester, para-toluene sulphonic acid salt (120g, 0.3M) was dissolved in dry methanol (300ml)

and the pH (moist pH paper) adjusted to ~6 using Et_3N and acetic acid. Methyl pyruvate (62.4g, 0.6M) in dry methanol (10.0ml) and 3A molecular sieves were added; the mixture was cooled to 5° and then $NaBH_3CN$ (100g, 1.58M) in methanol (600ml) added. After stirring for 3 days the reaction mixture was filtered and evaporated _in vacuo_. The residual white solid was partitioned between H_2O (500ml) and CH_2Cl_2 (4 x 200ml); the organic phase was evaporated to a yellow oil and then partitioned between hexane (250ml) and 1M oxalic acid (4 x 250ml). The aqueous phase was neutralised with $NaHCO_3$ and extracted into CH_2Cl_2 (4 x 250ml). The organic phase was dried and evaporated _in vacuo_ to yield a yellow oil (90g), which was chromatographed on SiO_2 using a gradient of EtOAc in hexane as eluant. The faster running diastereoisomer,

N-[1-(R)-methoxycarbonylethyl]-L-leucine benzyl ester, was isolated as an oil (22g, 20%); $[\alpha]_D^{20} = -49.5°$ (C=0.2, MeOH); (Found: C, 66.06; H, 8.19; N, 4.75. $C_{17}H_{26}NO_4$ requires C, 66.42; H, 8.19; N, 4.54); ν_{max} (nujol) 1735 cm^{-1}; $\delta(CDCl_3)$ 0.89 and 0.92 (each 3H, each d, each J=3.5 Hz., $(C\underline{H}_3)_2$); 1.29 (3H,d,J=7Hz; $C\underline{H}_3$); 1.5 (2H,m,$C\underline{H}_2CH$): 1.74 (2H,m,NH and CH_2 $C\underline{H}$ $(CH_3)_2$); 3.34 (1H,q, J=7Hz, $C\underline{H}CH_3$), 3.39 (1H,t,J=7Hz, $CH_2C\underline{H}$ (NH)CO); 3.69 (3H, s, OCH_3); 5.15 (2H,m,$OCH_2C_6H_5$) and 7.35 (5H,s,C_6H_5); m/e 308 (100%, $[M+1]^+$); 232 (53) and 172 (44).

The slow running diastereoisomer, N-[1-(S)-methoxycarbonylethyl)-L-leucine benzyl ester, was isolated as an oil (11.3g, 10%); $[\alpha]_D^{20}$ = 1.73° (C=0.2, MeOH); (Found: C, 66.42; H, 8.30; N, 4.54. $C_{17}H_{26}NO_4$ requires C, 66.42; H, 8.19; N, 4.55%) ν_{max} (film) 1730cm^{-1}; δ ($CDCl_3$) 0.87 and 0.9 (each 3H, each d, each J=5.5Hz, $(C\underline{H}_3)_2CH$); 1.27 (3H,d,J=7Hz, $C\underline{H}_3CH$); 1.49 (2H,m, $(CH_3)_2$-$CHC\underline{H}_2$); 1.74 (1H, heptet, J=7Hz, $(CH_3)_2C\underline{H}$); 2.2 br (1H,s,NH), 3.3 (2H,m,$C\underline{H}NHC\underline{H}$), 3.65 (3H,s,$OCH_3$); 5.13 (2H,s,$OCH_2C_6H_5$) and 7.35 (5H,s,$C_6H_5$); m/e 308 (100%, $[M+1]^+$) and 172 (100).

The R-benzyl ester (13g, 42mM) was dissolved in methanol (300ml) and hydrogenated over 10% palladium on charcoal at atmospheric pressure. The catalyst was removed by filtration through celite and the filtrate evaporated in vacuo to yield a white gum, which was crystallised from $MeOH/Et_2O$ to give the required leucine derivative as a white crystalline solid (7.5g, 82%); mp 150-151°; (Found: C, 55.27; H, 8.72; N, 6.43. $C_{10}H_{19}NO_4$ requires C, 55.3; H, 8.81; N, 6.45%); $[\alpha]_D^{20}$ 8.4 (C=0.2, MeOH); ν_{max}(nujol) 3400 br, 2500 br and 1755 cm^{-1}; δ(d^6DMSO) 0.85 (6H, m, $(C\underline{H}_3)_2CH_2$); 1.17 (3H,d,J=7Hz, $C\underline{H}_3CH$); 1.38 (2H,m, $(CH_3)_2CHC\underline{H}_2$); 1.74 (1H, heptet, J=6Hz, $(CH_3)_2C\underline{H}$); 3.14 (1H, t, J=7Hz, $NHCH(CH_2)CO_2H$); 3.29 (1H,q,J=7Hz, $CH_3C\underline{H}$) and 3.6 (3H, s,OCH_3); m/e 218 (100%, $[M+1]^+$), 172 (27) and 158 (17).

EXAMPLE 3

N-[2-N-[N-(2,4-Dinitrophenyl)-L-prolyl-L-leucyl]amino-1-(R)-methoxycarbonylethyl]-L-leucyl-O-benzyl-L-threonine N-Methylamide

This was prepared starting from methyl N-t-butyloxycarbonyl-N-benzyloxycarbonyl(R)-2,3-diaminopropionate and benzyl 4-methyl-2-oxo-pentanoate in the steps described in the following paragraphs.

(a) N-[2-N-(t-Butyloxycarbonyl)amino-1-(R)-methoxycarbonylethyl]-L-leucine Benzyl ester

To a stirred solution of methyl N-t-butyloxycarbonyl-N-benzyl oxycarboxyl-(R)-2,3-diaminopropionate (25g) in THF (150ml) and acetic acid (8ml) was added palladised charcoal (10%, 2g) and the mixture hydrogenated at 25° and 760 mmHg for 2h. The catalyst was removed by filtration and to the filtrate was added THF (50ml), benzyl 4-methyl-2-oxopentanoate (50g, from the corresponding acid by treatment at reflux with benzyl alcohol in the presence of para-toluene sulphonic acid and azeotropic removal of water) in THF (50ml) and finally water (70ml). The pH of the rapidly stirred solution was adjusted to 6.5 with triethylamine and sodium cyanoborohydride (4.5g) was added portionwise over 0.5h. The pH was maintained at 6.5 by periodic addition of acetic acid. After 16h at 20°, a further portion of sodium cyanoborohydride (2g) was added and stirring continued for 24h. The reaction mixture was concentrated *in vacuo* and the residue was partitioned be-

tween CH_2Cl_2 (200ml) and water (100ml). The aqueous layer was washed with fresh CH_2Cl_2 (2 x 100ml) and the combined organic extracts washed successively with 3N-citric acid solution water and finally saturated aqueous sodium hydrogen carbonate solution and then dried over $MgSO_4$. The oil isolated from the CH_2Cl_2 was purified by chromatography on silica eluting with CH_2Cl_2 in an increasing ethyl acetate gradient to give the required <u>benzyl ester</u> (9.6g) as an oil which slowly crystallised, m.p. 59.5-61° (from ether-hexane); $[\alpha]_D^{25}$ = 22.1° (C = 1.1, MeOH); (Found: C, 62.40; H, 8.08; N, 6.57. $C_{22}H_{34}N_2O_6$ requires C, 62.54; H, 8.11; N, 6.36%); ν_{max} ($CHCl_3$) 1730 and 1705 cm^{-1}; δ ($CDCl_3$) 0.89 (6H, t, J=6.3Hz, $CH(C\underline{H}_3)_2$); 1.43 (9H,s, $C(C\underline{H}_3)_3$); 1.50 (2H, m, $C\underline{H}_2CH$); 1.76 (2H,m,$CH_2C\underline{H}(CH_3)_2$ and NH); 3.35 (4H,m,CH_2N and 2x α-C$\underline{H}$); 3.67 (3H,s,OCH_3); 4.98 br (1H,s,NHCOO), 5.12 (2H,d, J=11.5Hz, CH_2Ph) and 7.36 (5H, m,C_6H_5); m/e 423 ($[M+1]^+$).

(b) <u>N-[2-N-(t-Butyloxycarbonyl)amino-1-(R)-methoxycarbonylethyl]-L-leucyl-O-benzyl-L-threonine N-Methylamide</u>

The foregoing benzyl ester (6g) in methanol (50ml) was hydrogenated at S.T.P. over 10% palladised charcoal (100mg) for 0.5h. The catalyst was removed by filtration and the material recovered from the methanol was recrystallised from methanol-ether to give the intermediate carboxylic acid (4.5g),

m.p. 147-148°. A portion of this material (2.8g) in CH_2Cl_2

(100ml) and DMF (20ml) was treated at 0° with 1-hydroxybenzotriazole(1.3g), and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.61g). After 0.5h at 0°, L-O-benzyl-threonine N-methylamide (1.86g) in CH_2Cl_2 (10ml) was added and the mixture allowed to warm to 20° over 1h. After 36h at 20°, the reaction mixture was washed in turn with saturated sodium hydrogen carbonate solution, 3N-citric acid solution and finally brine and then dried and evaporated *in vacuo*. Crystallisation of the resulting oil from ether-pentane gave N-[2-N-(t-butyloxycarbonyl)amino-1-(R)-methoxycarbonylethyl]-L-leucyl-O-benzyl-L-threonine N-methylamide (3g), m.p. 95-97°; (Found; C, 60.42; H, 8.20; N, 10.44. $C_{27}H_{44}N_4O_7$ requires C, 60.43; H, 8.26; N, 10.44%); δ ($CDCl_3$) 0.94 (6H,d, J=6.2Hz., $CH(C\underline{H}_3)_2$); 1.13 (3H,d,J=6.4Hz, $CHC\underline{H}_3$); 1.43 (9H,s,$C(C\underline{H}_3)_3$); 1.54-1.85 (3H,m,$C\underline{H}_2C\underline{H}$); 1.95 broad (1H,s, NH); 2.82 (3H,d, J=4.8Hz, $NHC\underline{H}_3$); 3.1-3.62 (4H,m,NCH_2, OC$\underline{H}$ and αC$\underline{H}$), 3.65 (3H,s,OCH_3), 4.3 (1H,m,α-C$\underline{H}$), 4.45 (1H,dd, J=6.3 and 2.3Hz, α-C$\underline{H}$); 4.54 and 4.62 (each 1H, each d, each J=11.6Hz, CH_2Ph); 5.05 broad (1H,s, NHCOO), 7.05 (1H,m,$N\underline{H}CH_3$), 7.32 (5H,m,C_6H_5) and 7.88 (1H,d, J=8.4Hz, NH).

(c) N-[2-N-[N-(2,4-Dinitrophenyl)-L-prolyl-L-leucyl]amino-1-(R)-methoxycarbonylethyl]-L-leucyl-O-benzyl-L-threonine N-Methylamide

To a stirred solution of the foregoing t-butyloxy-

carbonyl protected peptide (536mg) in CH_2Cl_2 (3ml) was added trifluoroacetic acid (3ml) at 0°. The solution was allowed to warm to 20° and then stirred at this temperature for 2h. The residue after evaporation of the organic solvents was taken into CH_2Cl_2 and the solution washed with saturated sodium hydrogen carbonate solution, dried (Na_2SO_4) and evaporated <u>in vacuo</u> to yield N-[2-amino-1-(R)-methoxycarbonylethyl]-L-leucyl-O-benzyl-L-threonine N-methylamide (330 mg). This material in CH_2Cl_2 (10ml) was added to a solution of N-[N-(2,4-dinitrophenyl)-L-prolyl]-L-leucine (330mg) in CH_2Cl_2 (10ml) containing 1-hydroxy-benzotriazole (132mg) and N-ethyl-N'-(3-dimethylamino-propyl)carbodiimide hydrochloride (191mg) stirred at 5°. After 16h at 4° the solvent was removed <u>in vacuo</u> and the residue in ethyl acetate washed in turn with water, saturated sodium hydrogen carbonate solution and finally 3N-citric acid solution. The material isolated from the ethyl acetate was recrystallised from CH_2Cl_2- ether to give the required <u>peptide</u> (440mg), m.p. 138-142°; (Found: C, 57.34; H, 6.92; N, 13.61. $C_{39}H_{56}N_8O_{11}$ requires C, 57.62; H, 6.94; N, 13.78%); ν_{max} ($CHCl_3$) 3295, 1730 and 1635 cms^{-1}; δ ($CDCl_3$) 0.95 (12H,m, 2xCH($C\underline{H}_3$)$_2$); 1.14 (3H,d,J=6.3Hz, $C\underline{H}_3$CH); 1.3-2.15 and 2.45 (10H,m,$C\underline{H}_2C\underline{H}_2$ and 2x$C\underline{H}_2C\underline{H}$); 2.74 (3H,s,$NHC\underline{H}_3$); 3.3 (3H,m,$CH_2N$ and CHO); 3.56 (3H,m,CH_2N and α-C$\underline{H}$); 3.63 (3H,s,OCH_3); 4.06, 4.25 and 4.56 (1H,2H and 1H respectively, each m, 4xαC$\underline{H}$); 4.43 and 4.55 (each

1H, each d, J=11.7Hz, CH_2Ph); 7.00 (1H,d, J=9.5Hz, 6-H in C_6H_3); 7.28 (5H,s,C_6H_5); 8.16 (1H,dd, J=9.5 and 2.8Hz, 5-H in C_6H_3) and 8.54 (1H,d, J=2.8Hz, 3-H in C_6H_3); m/e 813 ($[M+1]^+$).

O-Benzyl-l-threonine N-methylamide used in step (b) above was prepared from N-t-butyloxycarbonyl-O-benzyl-L-threonine N-methylamide by exposure to trifluoroacetic acid in CH_2Cl_2. This in turn was prepared from N-t-butyloxycarbonyl-O-benzyl-L-threonine and methylamine using the procedure described in Example 2 for the tyrosine analogue.

N-[N-(2,4-Dinitrophenyl)-L-prolyl]-L-leucine used as starting material in stage (c) was prepared from N-(2,4-dinitrophenyl)-L-proline and leucine methyl ester using the coupling procedure involving N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride as the condensing agent in the presence of 1-hydroxybenzotriazole (as illustrated in Example 3) followed by hydrolysis of the methyl ester with 2N-sodium hydroxide solution (see Example 5).

Methyl Nβ-t-butyloxycarbonyl-Nα-benzyloxycarbonyl-(R)-2,3-diaminopropionate, used as the starting material in stage (a), was prepared as follows:

To a stirred suspension of N-benzyloxycarbonyl-(R)-2,3-diaminopropionic acid [19.5g; from Nα-benzyloxycarbonyl-D-asparagine exactly as described for the L-isomer in

Synthesis, 266, (1981)] in dry methanol (60ml) at -20° was added thionyl chloride (30g) dropwise over 40min. The reaction mixture was allowed to warm to 20° over 1h and then heated at 50° for 1h. The residue after removal of the solvent was recrystallised from methanol-ether to give methyl Nα-benzyloxycarbonyl-R-2,3-diaminopropionate hydrochloride (22.5g); m.p. 170-172°; (Found: C, 49.86; H, 5.89; N, 9.53. $C_{12}H_{17}N_2O_4Cl$ requires C, 49.91; H, 5.93; N, 9.70%); ν_{max} (Nujol) 3305, 1735 and 1688 cm^{-1}; δ (d^6 DMSO) 3.00-3.32 (2H,m,CH_2NH_2); 3.7 (3H,s,OCH_3); 4.45 (1H,m,α-CH); 5.09 (2H,s,CH_2Ph); 7.36 (5H,s,C_6H_5); 7.95 (1H,d,J=7.5Hz, NHCOO) and 8.28 broad (3H,s, NH_3); m/e 253 ($[M+1]^+$). A portion of this material (22.5g) in DMF (150ml) was treated with Et_3N until the pH was 10. Di-t-butyl dicarbonate (16.8g) was added to the solution stirred at 5°. After a further 2h at 20°, the reaction mixture was filtered and evaporated in vacuo and the residue partitioned between ether and water. The aqueous layer was extracted twice more with fresh ether and the combined organic extracts washed in turn with ice cold 1N-hydrochloric acid, saturated sodium hydrogen carbonate solution and finally water. The oil isolated from the ether was crystallised from ethyl acetate-hexane to give methyl Nβ-t-butyloxycarbonyl-Nα-benzyloxycarbonyl-(R)-2,3-diaminopropionate (22.5g); m.p. 89-91°; (Found: C, 57.86; H, 6.95; N, 7.93. $C_{17}H_{24}N_2O_6$ requires C, 57.94; H, 6.86;

N, 7.95%); ν_{max} ($CHCl_3$) 3600 and 1700 cm^{-1}; δ 1.4 (9H, s, $C(C\underline{H}_3)_3$); 3.5 broad (2H, s, CH_2N); 3.72 (3H, s, OCH_3); 4.4 (1H, α-C$\underline{H}$); 5.09 (2H, s, CH_2Ph); 5.2 broad (1H, s, NHCOO); 6.06 (1H, d, J=7.3Hz, NHCOO) and 7.32 (5H, s, C_6H_5).

EXAMPLE 4

N-[1-(R)-Methoxycarbonylethyl]-L-leucyl-O-benzyl-L-tyrosine N-Methylamide

Leucine benzyl ester *para*-toluene sulphonic acid salt (113g) in dry acetonitrile (800ml) was treated with methyl 2-bromopropionate (62.7ml) and N-methyl morpholine (100ml) under reflux for 16h. The reaction mixture was concentrated *in vacuo* and the residue in ethyl acetate washed with brine, dried (Na_2SO_4) and evaporated. Chromatography of the resulting oil on silica in 1:4 ethyl acetate-hexane gave N-[1-(R)-methoxycarbonylethyl]-L-leucine benzyl ester (45g) as the faster running fraction. This material was treated exactly as described above in Example 2 to give the title compound.

EXAMPLE 5

N[1-(R)-Carboxyethyl]-L-leucyl-O-benzyl-L-tyrosine N-Methylamide

The methyl ester (1.5g, 3.1mM) from Example 2 was dissolved in methanol (20ml) and treated with 1N sodium hydroxide (3.5ml, 3.5mM). After 18h, the pH was adjusted to 5 with acetic acid and the solvent removed *in*

vacuo to yield a white solid. Recrystallisation first from water and then from methanol-ether yielded the N-[1-(R)-carboxyethyl]-L-leucyl-O-benzyl-L-tyrosine N-methylamide as a white powder (1.02g), m.p. 195°; $[\alpha]_D^{20} = +7.2°$ (C = 0.2, MeOH); (Found: C, 63.76; H, 7.64; N, 8.57. $C_{26}H_{35}N_3O_5 \cdot H_2O$ requires C, 64.05; H, 7.65; N, 8.62%); ν_{max} (Nujol) 3540 (br), 3330 and 1680 cm^{-1}; δ (d^6DMSO) 0.82 (3H,d, J=6Hz, $(C\underline{H}_3)_2CH$); 0.87 (3H,d, J=6Hz, $(C\underline{H}_3)_2CH$); 1.13 (3H,d, J=7Hz., $C\underline{H}_3CH$); 1.24 (2H,t, J=6Hz., $CHC\underline{H}_2CH$: 1.59 (1H,m, $(CH_3)_2C\underline{H}CH_2$); 2.63 (3H,d, J=5Hz, $NHC\underline{H}_3$); 2.72 (1H,dd, J=11 and 12Hz, $CHC\underline{H}_2C_6H_4$); 2.8 (1H,q, J=7Hz, $CH_3C\underline{H}(NH)CO_2H$); 2.95 (1H,dd, J=12 and 5Hz, $CHC\underline{H}_2C_6H_4$); 3.21 (1H,t, J=7.5Hz, $NHC\underline{H}(CH_2CH(CH_3)_2CO)$; 4.53 br (1H,m, $NHC\underline{H}(CH_2C_6H_4)CO)$; 5.08 (2H,s,$C_6H_4OC\underline{H}_2C_6H_5$); 6.93 and 7.17 (each 2H, each d, each J=7.5Hz, C_6H_4O); 7.48 (5H,m,C_6H_5); 7.98 br (1H,q, J=5Hz, $N\underline{H}CH_3$, exch) and 8.1 (1H,d, J=9Hz., $CHCON\underline{H}CH$, exch); m/e 470 (88% $[M+1]^+$), 452 (51), 424 (29), 285 (100) and 158 (49).

Example 6

N[1-(R)-Carboxyethyl]-L-Leucine N-Phenethylamide

N[1-(R)-Ethoxycarbonylethyl]-L-Leucine N-phenethylamide (710mg, 2.1mM) was dissolved in MeOH (50ml) and treated with 1N NaOH (3ml, 3mM) at room temperature. After 12h, the reaction mixture was acidified with AcOH and evaporated *in vacuo* to a solid which was washed with H_2O and dried to yield the title compound (400mg); m.p. 201-205°; (Found: C,66.44; H,8.55; N,9.11; $C_{17}H_{26}N_2O_3$ requires C,66.64; H,8.55; N,9.14%); ν_{max} (Nujol) 3330, 1660 and 1530 cm^{-1}; δ(d^6DMSO) 0.825 (6H,t,J=6.2Hz, $(C\underline{H}_3)_2CH$), 1.15 (3H,d, J=6.8Hz, $C\underline{H}_3CH$), 1.29 (2H,m,$C\underline{H}_2CH$), 1.55 (1H, heptet,J=7Hz, $C\underline{H}(CH_3)_2$), 2.71 (2H,t,J=7Hz, $C\underline{H}_2C_6H_5$), 3.0 (1H,q,J=7Hz, $C\underline{H}CH_3$), 3.14 (1H,t,J=7Hz,α-C$\underline{H}$), 3.32 (2H,q,J=6Hz, $NHC\underline{H}_2CH_2$), 7.12 (5H,m,C_6H_5), 7.5 (2H,br s,O$\underline{H}$ and CHN$\underline{H}$CH) and 8.15 (2H,t,J=5Hz, N$\underline{H}$$CH_2$).

The N[1-(R)-ethoxycarbonylethyl]-L-leucine N-phenethylamide required in the preparation above was synthesised as follows:

N[1-(R)-Ethoxycarbonylethyl]-L-leucine (1.39g, 6mM), N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1.16g, 6mM), 1-hydroxybenzotriazole (0.93g, 6mM) and phenethylamine (0.7g, 6mM) were dissolved in DMF (50ml) at -6°. N-Methyl-morpholine (0.62g, 6.2mM) was added and the reaction mixture allowed to warm to room temperature. After 12h the

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solvent was removed *in vacuo*. The residue in EtOAc (150ml) was washed with H_2O (2 x 100ml), dried and evaporated *in vacuo* to yield an oil which was purified by chromatography on SiO_2 in EtOAc to give N[1-(R)-ethoxycarbonylethyl]-L-leucine N-phenethylamide as an oil (1.84g). For analysis, a portion of this material was dissolved in MeOH, treated with anhydrous HCl in Et_2O and evaporated *in vacuo* to yield the corresponding hydrochloride as a foam; (Found: C,60.28; H,8.54; N,7.35; $C_{19}H_{30}N_2O_3$.HCl.0.4 H_2O requires C,60.35; H,8.48; N,7.41%); $[\alpha]_D^{20} = +9.4^\circ$ (c=0.2, MeOH); ν_{max} (Nujol) 3400 (br), 3100 (br), 2510 (br), 2400 (br), 1740, 1670 and 1550 cm^{-1}; δ ($CDCl_3$) 0.92 and 0.95 (6H, each d, each J=7Hz, $(C\underline{H}_3)_2CH$), 1.27 (3H, t, J=6.5Hz, $C\underline{H}_3CH_2$), 1.28 (3H,d,J=7Hz, $C\underline{H}_3CH$), 1.3-1.7 (3H,m,CH_2CH), 2.85 (2H,t,J=6Hz,$CH_2C\underline{H}_2C_6H_5$), 3.21 (1H,dd,J=10Hz and 4Hz,α-$C\underline{H}$), 3.27 (3H,q,J=6.5Hz, $CH_3C\underline{H}$), 3.54 (2H,q,J=7Hz, $NHC\underline{H}_2CH_2$), 4.16 and 4.18 (2H, each q, each J=6.5Hz, $OC\underline{H}_2CH_3$) and 7.25 (6H,m,C_6H_5 and NHCO).

The starting material required in the preparation given above was synthesised in two steps from leucine benzyl ester as follows:

(a) <u>N[1-(R)-Ethoxycarbonylethyl]-L-Leucine Benzylester</u>

L-Leucine benzyl ester (186.65g, 0.843M), ethyl 2-bromopropionate (153.1g, 0.846M) and N-methylmorpholine (165ml, 1.5M) were dissolved in dry CH_3CN (600ml) and refluxed for 12h. The solvent was

removed in vacuo and the residue partitioned between H_2O (2l) and EtOAc (3 x 1l). The organic phase was washed with brine, dried and evaporated *in vacuo*. The resulting oil was chromatographed on SiO_2 in 7.5% EtOAc in hexane to give the *title compound* (70g) as the faster running fraction; (Found: C,67.02; H,8.42; N,4.25; $C_{18}H_{27}NO_4$ requires C,67.26; H,8.47; N,4.36%); ν_{max} film 1730cm^{-1}; $\delta(CDCl_3)$ 0.88 and 0.9 (each 3H, each d, each J=6.5Hz, $CH(C\underline{H}_3)_2$) 1.23 (3H,t,J=7Hz,$C\underline{H}_3CH_2O$), 1.27 (3H,d,J=7Hz,$C\underline{H}_3CH$), 1.5 (2H,m,$CHC\underline{H}_2$), 1.7 (1H, heptet, J=7Hz, $C\underline{H}(CH_3)_2$), 2.2 (1H, br s,N$\underline{H}$), 3.32 (2H,m, $C\underline{H}NHC\underline{H}$), 4.10 and 4.12 (each 1H, each q, each J=7Hz, $OC\underline{H}_2CH_3$), 5.12 (2H,s, $OC\underline{H}_2C_6H_5$) and 7.34 (5H,s, C_6H_5); m/e 322 (100%; $[m+1])^+$, 260 (15), 186 (26) and 112 (28).

(b) N[1-(R)-Ethoxycarbonylethyl]-L-leucine

N[1-(R)-Ethoxycarbonylethyl]-L-leucine benzyl ester (69.09g, 0.215M) was dissolved in MeOH (300ml) and hydrogenated at 1 atmosphere over 5% palladium on charcoal (5g). After 1.5h, the catalyst was removed by filtration and the filtrate evaporated *in vacuo* to yield a solid (46.8g) which was recrystallised from $MeOH/Et_2O$ to yield the *title compound* (24g); m.p. 149-150^o; $[\alpha]_D^{20}$=8.8^o (C=1.4, MeOH); (Found: C,57.14; H,9.06; N,6.02; $C_{11}H_{21}NO_4$ requires C,57.12; H,9.15; N,6.06%); ν_{max} (Nujol) 3090 (br), 2300 (br), 1755 and 1560 cm^{-1}; δ(d^{6}DMSO) 0.86 and 0.87 (6H, each d, each J=6.5Hz, $(C\underline{H}_3)_2CH$), 1.19 (6H,m,$OCH_2C\underline{H}_3$ and $CHC\underline{H}_3$), 1.36 (2H,m,

$CHCH_2CH$), 1.74 (1H, heptet, J=6.5Hz, $CH_2CH(CH_3)_2$), 3.14 (1H,t,J=6.2Hz,α-CH), 3.27 (1H,q,J=6.8Hz,$NHCHCH_3$) and 4.07 (2H,q,J=7Hz,OCH_2CH_3); m/e 232 (100%, $[m+1]^+$), 186 (3) and 158 (7).

Example 7

N[1-(R)-Carboxy-3-methylthiopropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

This was prepared from D-methionine methyl ester hydrochloride, 2-oxo-4-methylpentanoic acid and O-methyl-2-tyrosine in the steps described in the following paragraphs.

(a) N[1-(R)-Carbomethoxy-3-methylthiopropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

D-Methionine methyl ester hydrochloride (10g, 50mM) and 2-oxo-4-methylpentanoic acid t-butyl ester (9.3g, 50mM) were dissolved in THF (75ml) and H_2O (25ml). The pH was adjusted to 6.5 with N-methylmorpholine, $NaCNBH_3$ (630mg, 10mM) was added, followed after 2h by a further portion (400mg). After 18h the reaction mixture was evaporated *in vacuo* and then partitioned between EtOAc (100ml) and sat.$NaHCO_3$ solution (2x100ml). The oil isolated from the organic layer was chromatographed on SiO_2 using a gradient of 5-10% EtOAc in hexane. The faster running fraction afforded the required isomer as an oil (1.4g). δ($CDCl_3$) 0.96 (6H,m, $(CH_3)_2CH$), 1.5 (9H,m, $(CH_3)_3C$), 1.4-2.0 (5H,m, CH_2CH and

SCH_2CH_2CH), 2.1 (3H,S,CH_3S), 2.62 (2H,m,SCH_2CH_2), 3.17 and 3.38 (each 1H, t, each J=7Hz, CHNHCHØ and 3.7 (3H,s,OCH_3)]. The slower running isomer was also obtained as an oil (1.2g). δ ($CDCl_3$) Ø.95 (6H,m, $(CH_3)_2CH$), 1.5 (9H,s, $(CH_3)_3C$), 1.5-2.1 (5H,m, SCH_2CH_2CH and CH_2CH), 2.Ø9 (3H,s,CH_3S), 2.6 (2H,m, SCH_2CH_2), 3.Ø8 and 3.22 (each 1H, each dd, each J=7Hz, CHNHCH) and 3.7 (3H,s,OCH_3)].

The faster running t-butyl ester (2.9g, 9mM) from above was dissolved in TFA (5Øml) and H_2O (Ø.5ml). After 3h the mixture was evaporated *in vacuo*, toluene (5Øml) was added and the solution was reevaporated *in vacuo*. The resulting oil was dissolved in CH_2Cl_2 (1ØØml) and the pH adjusted to 7 (moist pH paper). O-Methyl-L-tyrosine N-methylamide (2.Øg, 1ØmM) and 1-hydroxybenzotriazole (1.5g, 1ØmM) were added. The reaction mixture was cooled to Ø^o, treated with dicyclohexylcarbodiimide (2.1g, 1ØmM) and then allowed to warm slowly to room temperature. After 18h, the mixture was filtered and the filtrate washed with H_2O and sat. $NaHCO_3$ solution. After drying, the solvent was removed *in vacuo* to yield an oil which was chromatographed on SiO_2 in 1:1 EtOAc/hexane. The relevant fractions yielded, after recrystallisation from Et_2O/hexane, the *title compound* (1.4g); m.p. 1Ø8-111°; (Found: C,58.62; H,7.91; N,8.85; $C_{23}H_{35}N_3O_5S$ requires C,59.Ø7; H,7.97; N,8.96%); ν_{max} (Nujol) 338Ø

(br), 1740, 1610 and 1560 cm^{-1}; δ ($CDCl_3$) 0.86 and 0.87 (each 3H, each d, each J=6Hz, $(C\underline{H}_3)_2CH$), 1.15 and 1.4 (each 1H, each m, $C\underline{H}_2CH(CH_3)_2$), 1.6 (1H,m,CH), 1.90 (2H,m,$SCH_2C\underline{H}_2$), 2.08 (3H,s,CH_3S), 2.5 (2H,m, $SC\underline{H}_2$), 2.77 (3H,d,J=6Hz, $NHC\underline{H}_3$), 3.05 (3H,m,$C\underline{H}_2C_6H_5$ and α-CH) 3.47 (1H,t,J=5Hz, α-C$\underline{H}$), 3.7 (3H,s,$OC\underline{H}_3$), 3.8 (3H,s,CO_2CH_3), 4.63 (1H,q,J=7Hz, α-C$\underline{H}$), 6.69 (1H,brq,J=6Hz, $N\underline{H}CH_3$), 6.82 and 7.13 (each 2H, each d, J=9Hz,C_6H_4) and 7.53 (1H,d,J=9Hz, CON$\underline{H}$CH); m/e 468 (100%, $[m+1]^+$) and 232 (27).

(b) <u>N[1-(R)-Carboxy-3-methylthiopropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide</u>

N[1-(R-)-Carbomethoxy-3-methylthiopropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide (100mg, 0.2mM) was dissolved in MeOH (10ml) and treated with IN NaOH (0.25ml, 0.25mM). After 18h another portion of IN NaOH (0.5ml, 0.5mM) and H_2O (2ml) were added. After a further 18h the reaction mixture was acidified with AcOH and evaporated *in vacuo*. The resulting white solid was chromatographed on C_{18}-Silica eluting with a gradient of 10% to 40% MeOH in H_2O. The relevant fractions were pooled and evaporated *in vacuo*; the residue was recrystallised from hot H_2O to yield the <u>title compound</u> (20mg); m.p. 170-180; (Found: C,56.88; H,7.49; N,9.05; calculated for $C_{22}H_{35}N_3O_5S,.0.6H_2O$: C,56.90; H,7.86; N,9.0 5%); ν_{max} (Nujol) 3340, 1650 and 1625 cm^{-1}; δ (d^6DMSO) 0.82 (6H,t,J=7Hz, $(C\underline{H}_3)_2CH$), 1.17 and

1.5-1.9 (5H,m, $CH_2CH(CH_3)_2$ and SCH_2CH_2), 2.04 (3H,s,CH_3S), 2.3 (2H,m,-SCH_2), 2.6 (3H,d,J=5Hz, $NHCH_3$), 2.6-2.95 (3H,m,$CH_2C_6H_4$ and α-CH),), 3.14 (1H,t,J=7Hz,α-CH),3.7 (3H,s,OCH_3), 4.25 (1H,m, -CH), 6.8 and 7.12 (2x2H, each d, each J=9Hz,C_6H_4), 7.88 (1H,q,J=5Hz, $NHCH_3$) and 8.18 (1H,d,J=9Hz, NHCH); m/e 454 (100%, $[m+1]^+$).

O-Methyl-L-tyrosine N-methylamide used in stage (a) above was prepared from Z-L-tyrosine as follows:

(i) Z-L-Tyrosine-O-methyl ether

Z-L-Tyrosine (150g, 0.476 M) was dissolved with stirring in dilute aqueous sodium hydroxide (42g, 1.05M in 750ml H_2O). Dimethyl sulphate (51ml, 0.54 M) was then added dropwise over 30 min. to this solution at room temperature. After 2h further NaOH was added (4.2g, 0.105 M in 40ml H_2O) followed by dimethyl sulphate (5.1ml) after which the reaction was allowed to stir overnight at room temperature. The reaction was then acidified to pH 2, extracted with CH_2Cl_2 and the CH_2Cl_2 layer washed with aqueous sodium chloride, dried ($MgSO_4$) and concentrated *in vacuo* to yield the crude product. Recrystallisation from ethyl acetate/hexane gave the required methyl ether (155g); m.p. 114-115°; (Found: C,65.84; H,5.82; N,4.22. $C_{18}H_{19}NO_5$ requires C,65.64; H,5.81; N,4.25%); ν_{max} ($CHCl_3$) 3412 and 1715 cm^{-1}; δ ($CDCl_3$) 3.1 (2H,m, $CH_2C_6H_4$); 3.76 (3H,s,OCH_3); 4.66 (1H,dd,J=8 and 3Hz, α-CH); 5.1 (2H,m,$CH_2C_6H_5$);

5.23 (1H,d,J=8Hz, NH); 6.8 (2H,d,J=8.6Hz,Tyr H-3,H-5); 7.05 (2H,d,J=8.6 Hz,Tyr H-2,H-6); 7.33 (5H, broad s,C_6H_5); m/e 330 (68% $[M+1]^+$), 285 (100% $[M-CO_2H]^+$).

(ii) N-(Benzyloxycarbonyl)-O-methyl-L-tyrosine N-Methylamide

To a stirred solution of N-(Benzyloxycarbonyl)-O-methyl-L-tyrosine (155g, 0.471M) in dry CH_2Cl_2 was added 1-hydroxybenzotriazole (63.6g, 0.471 M) followed by a solution of DCC (97.2g, 0.471M) in CH_2Cl_2 (100 ml) added slowly at 0^oC. After warming to room temperature over 1hr, a solution of methylamine (30g) in CH_2Cl_2 (250ml) was added dropwise to the reaction mixture which was then stirred overnight at room temperature. The reaction was then filtered, washed with saturated aqueous sodium bicarbonate (x2), dried ($MgSO_4$) and concentrated *in vacuo* to give a solid. Recrystallisation from ethyl acetate/hexane gave the desired amide (142g); m.p. 167-170°; (Found: C,66.72; H,6.58; N,8.29. $C_{19}H_{22}N_2O_4$ requires C,66.65; H,6.48; N,8.18%) ν_{max} ($CHCl_3$) 3440, 1710 and 1672 cm^{-1}; δ($CDCl_3$) 2.70 (3H,d,J=5Hz,NCH_3); 2.98 (2H,m,$CH_2C_6H_4$); 3.77 (3H,s,OCH_3); 4.30 (1H,dd,J=7.6 and 3Hz, α-CH); 5.06 (2H,m,$OCH_2C_6H_5$); 5.43 (1H,m,OCONH); 5.84 (1H,m,CONH); 6.80 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.15 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.32 (5H,m,C_6H_5); m/e 343 (100%, $[m+1]^+$).

(iii) O-Methyl-L-tyrosine N-Methylamide

To a solution of N-(Benzyloxycarbonyl)-O-methyl-L-tyrosine N-methylamide (15.6g, 0.056 M) in ethanol (200ml) and DMF (200ml) was added 10% Pd/C (1g) and trifluoroacetic acid (4ml). Hydrogen was then passed through the solution for 3h after which the reaction was filtered and concentrated *in vacuo*. The residue was dissolved in H_2O (150ml), neutralised with sodium bicarbonate and extracted into CH_2Cl_2 (150ml x 5). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to yield an oil which subsequently crystallised. Recrystallisation from ethyl acetate/hexane gave O-methyl-L-tyrosine N-methylamide (9.0g), m.p. 90-91^{o}; (Found: C,63.49; H,7.71; N,13.44 $C_{11}H_{16}N_2OA$ requires C,63.44; H,7.74; N,13.45%) ν_{max} ($CHCl_3$) 3350 and 1660cm^{-1}; δ ($CDCl_3$) 1.3 (2H,br,N$\underline{H}_2$); 2.64 (1H,dd,J=13.8 and 9.2Hz,C$\underline{H}C_6H_4$); 2.80 (3H,d,J=5Hz,NC$\underline{H}_3$); 3.18 (1H,dd,J=13.8Hz and 4Hz, C$\underline{H}C_6H_4$); 3.55 (1H,dd,J=9Hz and 4Hz, α-C$\underline{H}$) 3.78 (3H,s,OC$\underline{H}_3$); 6.85 (2H,d,J=8,2Hz,Tyr H-3 and H-5); 7.12 (2H,d,J=8,2Hz, Tyr H-2 and H-6); 7.28 (1H,br,CONH).

Example 8

N-[4-N-(benzyloxycarbonyl)amino-1-(R)-methoxycarbonyl-butyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a solution of methyl 5-N-(benzyloxycarbonyl)amino-2-bromo-pentanoate (10.3g, 0.03 M), L-leucyl-O-methyl-L-tyrosine N-methylamide (9.6g, 0.03M)

and N-methyl morpholine in dry acetonitrile (100ml) was added sodium iodide (4.5g, 0.03 M). The mixture was then stirred and heated under reflux for 24hr. The cooled reaction mixture was then filtered and evaporated _in vacuo_ to yield an oil. Chromatography on silica eluting with dichloromethane in an increasing ethyl acetate gradient gave the _title compound_ (2.8g); m.p. 124-127°; (Found: C,63.7; H,7.52; N,9.56. $C_{31}H_{44}N_4O_7$ requires C,63.68; H,7.58; N,9.58%); ν_{max} ($CHCl_3$) 3400, 1718 and 1660 cm^{-1}; δ($CDCl_3$) 0.85 and 0.87 (each 3H, each d, each J=6Hz, CH)$C\underline{H}_3)_2$) 1.0-1.85 (8H,m,$NHCH_2C\underline{H}_2CH_2$, $C\underline{H}_2C\underline{H}$ and $N\underline{H}$); 2.74 (3H,d,J=5Hz,NCH_3); 2.96-3.42 (6H,m,$NHC\underline{H}_2$,α-C$\underline{H}$ x 2, $C\underline{H}_2C_6H_4$); 3.66 (3H,s,OCH_3); 3.75 (3H,s,OCH_3); 4.6 (1H,dd,J=13Hz and 6Hz, α-C$\underline{H}$); 5.0 (1H,m,OCONH); 5.1 (2H,s,$CH_2C_6H_5$); 6.71 (1H,br,CONH); 6.80 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.10 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.35 (5H,m,C_6H_5); 7.56 (1H,m,CON$\underline{H}$); m/e 585 (100% $[m+1]^+$).

The starting materials used in this preparation were synthesised as follows:

(a) L-Leucyl-O-methyl-L-tyrosine N-methylamide

To a solution of BOC-L-Leucine (5.26g, 0.021 M) in CH_2Cl_2 (40ml) and DMF (10ml) stirred at 0° was added N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (4g, 0.021 M). After 15 min., N-methyl morpholine (0.021 M) was added followed by, after a

further 10 min. at 0^o, a solution of O-methyl-L-tyrosine N-Methylamide (4.3g, 0.019 M) in CH_2Cl_2. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was then concentrated *in vacuo*, and the residue in CH_2Cl_2, washed in turn with H_2O (200ml), saturated aq. $NaHCO_3$ (200ml), dilute HCl (1M; 200ml), saturated aq. $NaHCO_3$ (200ml) and water (150ml), dried (Na_2SO_4) and evaporated *in vacuo* to a solid. Recrystallisation from ethyl acetate/hexane gave N-(Tertiarybutoxycarbonyl)-L-leucyl-O-methyl-L-tyrosine N-methylamide as a white crystalline solid, (4.5g), m.p. 159-161; (Found: C,62.65; H,8.33; N,9.96. $C_{22}H_{35}N_3O_5$ requires C,62.69; H,8.37; N9.97%). ν_{max} ($CDCl_3$) 3400, 1700 and 1662 cm^{-1}; δ($CDCl_3$) 0.91 (6H,dd,J=7 and 14Hz, $CH(CH_3)_2$); 1.37 (9H,s, $OC(C\underline{H}_3)_3$); 1.47-1.7 (3H,m,$C\underline{H}_2C\underline{H}(CH_3)_2$); 2.71 (3H,d,J=4.7Hz, $NHC\underline{H}_3$), 2.98 and 3.14 (each 1H, each m, $C\underline{H}_2C_6H_4$); 3.78 (3H,s,OCH_3); 4.0 and 4.61 (each 1H, each m, 2 x α-C$\underline{H}$); 4.86, (1H,br s,OCONH); 6.40 and 6.55 (each 1H, each br s, CONH x 2); 6.82 (2H,d,J=8.4Hz, Tyr H-3 and H-5); 7.08 (2H,d,J=8.4Hz, Tyr H-2 and H-6); m/e 422 (70%, $[m+1]^+$), 365 (70%, $[m-58]^+$).

To a stirred solution of N-Tertiarybutoxycarbonyl)-L-leucyl-O-methyl-L-tyrosine N-methylamide (7.0g, M) in CH_2Cl_2 (40ml) cooled at 10^o was added trifluoroacetic acid (70ml) and the resulting solution stirred at room temperature for 1h. The

reaction was then concentrated *in vacuo*, and the residue dissolved in water, neutralised with sodium bicarbonate and extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give L-leucyl-O-methyl-L-tyrosine N-methylamide (5.2g); m.p. 128-132°; (Found: C,60.04; H,8.72; N,12.26 $C_{17}H_{27}N_3O_3$ requires C,60.16 ; H,8.61; N,12.38 %); ν_{max} ($CDCl_3$) 3325 and 1655 cm^{-1}; $[\alpha]_D^{20}$ = 10.2° (C=2.00, MeOH); δ (CD_3OD) 0.88 and 0.92 (each 3H, each d; 1.2-1.4 (1H,m,$CH_2CH(CH_3)_2$); 1.44-1.8 (2H,m,$CHCH(CH_3)_2$); 2.73 (3H,d,J=5Hz, NCH_3); 2.82-3.3 (4H,m,NH_2,$CH_2C_6H_4$); 3.46 (1H,m,CH); 3.76 (3H,s,OCH_3); 4.58 (1H,q,dd,J=8 and 3Hz,α-CH); 6.56 (1H,br,CONH); 6.82 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.13 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.96 (1H,d,J=8Hz,CONH); m/e 322 (100% $[m+1]^+$).

(b) Methyl 5-N-(Benzyloxycarbonyl)amino-2-bromopentanoate

To a stirred solution of ε-Z-ornithine (53.2g, 0.1M) in dilute H_2SO_4 (2.5N, 500ml) at 0° was added KBr (60g, 0.5 M). To this solution was then added portionwise sodium nitrite (7.6g, 0.11 M) whilst the reaction temperature was maintained at 0° by the addition of ice. After stirring for 1h at 0° the reaction mixture was allowed to warm to room temperature over 2h. Diethyl ether (500ml) was then added and the aqueous layer was re-extracted with diethylether (500,; x 3). The combined ethereal extracts, were washed with water and

then brine, dried ($MgSO_4$), filtered and concentrated to an oil *in vacuo*.

To the crude bromo-acid (45g, 0.136 M) in dry methanol (300ml) cooled to -30° was added dropwise thionyl chloride (33.7ml, 0.405 M) at such a rate that the temperature did not exceed -15°. The reaction mixture was warmed to 10° over 2h and stirred at room temperature for 30 min. and then at 40° for 30 min. The resulting solution was then concentrated *in vacuo*, dissolved in CH_2Cl_2 and washed in turn with water, saturated aq. $NaHCO_3$ and water. The residue isolated from the organic layer was chromatographed on silica in 5% ethylacetate in CH_2Cl_2 to give the *title compound* as an oil (10.3g), (Found: C,48.61; H,5.61; N,4.00. $C_{14}H_{18}BrNO_4$ requires C,48.85; H,5.27; N,4.07%); δ($CDCl_3$) 1.5-1.8 and 1.9-2.2 (each 2H, each m, CH_2CH_2), 3.23 (2H,q,J=6Hz,NCH_2), 3.77 (3H,s,OCH_3), 4.25 (1H,dd,J=7 and 14Hz,α-CH), 4.8-4.9 (1H, broad s, NH), 5.10 (2H,s,OCH_2) and 7.35 (5H, broad s, C_6H_5).

Example 9

N-[4-N-(Benzyloxycarbonyl)amino-1-(R)-carboxybutyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a solution of the ester (650mg, 1.14 M) from Example 8 in methanol/water (10:1, 11ml) was added dilute NaOH (1N, 2.3ml). The reaction mixture was stirred for 6h at room temperature, acidified with

acetic acid and then concentrated to a semi-solid *in vacuo*. This was partitioned between ethyl acetate and water and the resulting solid was filtered, washed with water and ethyl acetate and dried *in vacuo* to give the *title compound* (585mg); m.p. 164-169°; (Found: C,61.59; H,7.24; N,9.40. $C_{30}H_{42}N_4O_7$ requires C,61.21; H,7.53; N,9.52%); ν_{max} (Nujol) 3320, 1690 and 1645 cm^{-1}; δ (d^6DMSO) 0.85 (6H,m,$CH(CH_3)_2$); 0.96-1.8 (7H,m,$CH_2CH(CH_3)_2$, $NHCH_2CH_2CH_2$); 2.57 (3H,d,J=5Hz,NCH_3); 2.5-3.2 (6H,m,$NHCH_2$, $CH_2C_6H_4$, α-CHx2); 3.70 (3H,s,OCH_3); 4.42 (1H,m,αCH); 5.0 (2H,s,$CH_2C_6H_5$); 6.78 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.10 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.20 (1H,m,CONH); 7.35 (5H,m,C_6H_5); 7.88 (1H,m,CONH); 8.18 (1H,m,CONH).

Example 10

N-[4-N-[N-(Acetyl)-L-prolyl-L-leucyl]amino-1-(R)-carboxybutyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

This was synthesised from Z-proline, leucine methyl ester and N-[4-N-(benzyloxycarbonyl)amino-1-(R)-methoxycarbonylbutyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide as described in the following paragraphs:

(a) N-(Benzyloxycarbonyl)-L-prolyl-L-leucine Ethyl Ester

To a stirred solution of Z-L-proline (12.7g, 0.051M) in CH_2Cl_2 (200 ml) cooled to 0° was added 1-hydroxy benzotriazole (7.0g) followed by a solution of DCC (10.6g) in CH_2Cl_2 (50 ml). After 30 min. at 0°

L-leucine ethyl ester (10.0g, 0.051 molM) was added followed by triethylamine (15 ml) and the reaction mixture was then left to stir and warm up to room temperature overnight. The reaction mixture was then filtered and washed in turn with saturated aq. $NaHCO_3$ (250 ml x 3), H_2O (250 ml), dilute aq. HCl (1M, 250 ml x 3) and water (250 ml x 2). The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo to an oil which subsequently crystallised. Recrystallisation from ethyl acetate/hexane gave N-(benzyloxycarbonyl)-L-prolyl-L-leucine ethyl ester as a white crystalline solid, 15.5g, (78%); m.p. 67-68^{o}; (Found: C,64.55; H,7.79; N,7.22. $C_{21}H_{30}N_2O_5$ requires C,64.61; H,7.74; N,7.17%); ν_{max} ($CHCl_3$) 1740 and 1680cm^{-1}; δ ($CDCl_3$) 0.7-0.95 (6H,m,$CH(C\underline{H}_3)_2$); 1.18 (3H,m,$OCH_2C\underline{H}_3$); 1.3-1.95 and 2.05-2.25 (7H,m,CH_2CH_2, $C\underline{H}_2CH(CH_3)_2$); 3.4 (2H,m,$CH_2N$); 4.05 (2H,m,$OC\underline{H}_2CH_3$); 4.25 (2H,m,$\alpha$-C$\underline{H}$); 4.98 and 5.05 (together 2H, respectively q,J=7Hz, and m, $C\underline{H}_2C_6H_5$); 7.35 (5H, broad s, C_6H_5) and 8.26 (1H,m,CONH); m/e 391 (100%, $[m+1]^+$).

(b) N-Acetyl-L-proline-L-leucine Ethyl Ester

To a solution of N-(benzyloxycarbonyl)-L-prolyl-L-leucine ethyl ester (7.5g, 0.02 mM) in methanol (100 ml) was added acetic acid and 10% Pd/C (0.8g). After stirring under hydrogen for 3h at room temperature the reaction was filtered and concentrated to an oil in vacuo. Trituation of the residue with ether and

recrystallisation from ethyl acetate/hexane gave L-prolyl-L-leucyl ethyl ester as the acetate salt (5.0g), m.p. 87-89°. ν_{max} 1760 and 1660 cm^{-1}; $\delta(CDCl_3)$ 0.94 (6H,m,$CH(C\underline{H}_3)_2$); 1.27 (3H,t,J=7Hz,$OCH_2C\underline{H}_3$); 1.45-2.35 (7H,m,$C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2C\underline{H}(CH_3)_2$); 2.2 (3H,s,$CH_3CO_2$); 3.1 (2H,m,$CH_2N$); 4.15 (1H,m,$\alpha$-C$\underline{H}$); 4.19 (2H,q,J=7Hz, $OC\underline{H}_2CH_3$); 4.55 (1H,m,α-C$\underline{H}$); 7.24 (2H,br,NH,CO_2H); 7.87 (1H,d,J=7Hz,CONH); m/e (100% $[m+1]^+$)].

To a solution of the foregoing amine (3.0g, 11.7mM) in CH_2Cl_2 (50ml) was added p-nitrophenylacetate (2g, 12MM). After stirring the reaction mixture at room temperature for 3 days it was diluted with CH_2Cl_2 (350ml), washed with water, dried (Na_2SO_4) and concentrated to an oil *in vacuo*. Chromatography on silica in 1:1 CH_2Cl_2/EtOAc followed by 9:1 CH_2Cl_2/MeOH yielded N-acetyl-L-prolyl-L-leucine ethyl ester as a pale yellow oil (2.2g); (Found $[m+1]^+$= 299.19704. $C_{15}H_{27}N_2O_4$ requires $[m+1]^+$=299.19707); ν_{max} $(CHCl_3)$ 3600-3100 (broad), 1735, 1675 and 1625 cm^{-1}; $\delta(CDCl_3)$ 0.95 (6H,m,$CH(C\underline{H}_3)_2$); 1.25 (3H,t,J=7Hz,$OCH_2C\underline{H}_3$); 1.44-2.5 (7H,m,$C\underline{H}_2C\underline{H}_2$, $C\underline{H}_2C\underline{H}(CH_3)_2$); 2.12 (3H,s,$CH_3CO$); 3.36-3.7 (2H,m,$CH_2N$); 4.18 (2H,t,J=7Hz,$OC\underline{H}_2CH_3$); 4.25-4.55 (1H,m, C$\underline{H}$ Pro); 4.6 (1H, C$\underline{H}$ Leu); 6.38 and 7.35 (1H, each d,J=7Hz,CONH).

(c) N-[4-N-[N-(Acetyl)-L-prolyl-L-leucyl]amino-1-(R)-methoxycarbonylbutyl]-L-leucyl-O-methyl-L-tyrosine

N-methylamide

To a solution of N-[4-N-(benzyloxycarbonyl)amino-1-(R)-methoxycarbonylbutyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide (570mg, 0.97 mM) in methanol (8ml) was added 10% Pd/C and dilute HCl (1M, 2ml). After stirring the reaction mixture under hydrogen for 2h at room temperature it was filtered and concentrated *in vacuo* to a solid, (490mg) (100%), which was used as such in the next step.

N-Acetyl-L-prolyl-L-leucine (271mg, 1.06 MM; obtained from the foregoing ethyl ester by hydrolysis in methanol with one equivalent of IN-sodium hydroxide solution at 20^{o} over 16h followed by neutralisation with dilute HCl) in CH_2Cl_2 (2ml) and DMF (2ml) was stirred at 0^{o} and 1-hydroxy benzotriazole (162mg, 1.06 mM) and N-ethyl-N'-(dimethylaminopropyl)carbodiimide hydrochloride (240mg, 1.06 mM) were then added. After 5 min N-methylmorphine (107mg, 1.06 mM) was added followed, after 15 min, by the amine hydrochloride (prepared above) (485mg, 0.96 mM). After stirring overnight at 0-4^{o}, the reaction mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 and washed in turn with water, saturated aq. $NaHCO_3$ and dilute HCl. The acid layer was separated, neutralised with $NaHCO_3$ and extracted with CH_2Cl_2. The organic extracts were dried, (Na_2SO_4) and evaporated *in vacuo* to yield the *title compound* as a foam (570mg); m.p. 68-72^{o}; (Found:

C,59.99; H,8.35; N,11.65. $C_{36}H_{56}N_6O_8.1H_2O$ requires C,59.98; H,8.39; N,11.66%); δ (d^6 DMSO) 0.82 (12H,m, $CH(C\underline{H}_3)_2$ x 2); 1.0-2.34 (14H,m,$C\underline{H}_2C\underline{H}_2$ x 2, $C\underline{H}_2C\underline{H}(CH_3)_2$x2); 1.98 and 2.0 (together 3H, each s, CH_3CO); 2.50-3.08 (8H,m,$C\underline{H}_2C_6H_4$, CH_2N x2 and 2x α-C$\underline{H}$); 2.56 (3H,d,J=5Hz,CH_3N); 3.54 (3H,s,OCH_3); 3.70 (3H,s,OCH_3); 4.0-4.5 (3H,m,α-C$\underline{H}$); 6.78 (2H,d,J=8Hz Tyr H-3 and H-5); 7.11 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.5-8.35 (4H,m,CONH).

(d) <u>N-[4-N-[N-(Acetyl)-L-prolyl-L-leucyl]amino-1-(R)-carboxybutyl]-L-leucyl-O-methyl-L-tyrosine</u> <u>N-Methylamide</u>

To a solution of the preceeding ester (380mg, 0.54mM) in methanol (5ml) was added dilute NaOH (1M, 1ml). After stirring overnight at room temperature, the reaction mixture was neutralised with acetic acid and concentrated *in vacuo*. Chromatography on reverse phase silica in 1:1 MeOH/H_2O gave the *title compound* (280mg); m.p. 97-101°; (Found: C,58.52; H,7.93; N,11.46. $C_{36}H_{56}N_6O_8.1.5H_2O$ requires C,58.72; H,8.31; N,11.74%). ν_{max} (Nujol) 3700-3140 (broad) and 1635 cm^{-1}; δ (CD_3OD) 0.9 (12H,M,2xCH($C\underline{H}_3$)$_2$); 1.4-2.25 (14H,M,2x$C\underline{H}_2CH_2$, 2x$CH_2C\underline{H}(CH_3)_2$); 1.98 and 2.0 (together 3H, each s, CH_3CO); 2.68 and 2.72 (together 3H, each s, CH_3N), 2.75-3.8 (8H,m,$C\underline{H}_2C_6H_5$, CH_2Nx2, 2x C$\underline{H}$); 3.75 (3H,s,OCH_3); 4.25-4.65 (3H,m,αC$\underline{H}$), 6.78 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.11 (2H,d,J=8.6Hz, Tyr H-2 and H-6).

Example 11

N-[3-N-(Benzyloxycarbonyl)amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

This was prepared in two stages from methyl 4-N-(benzyloxycarbonyl)amino-2-bromo-butanoate and L-leucyl-O-methyl-L-tyrosine N-methylamide as described below:

(a) N-[3-N-(Benzyloxycarbonyl)amino-1-(R)-methoxycarbonyl propyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

Methyl 4-N-(benzyloxycarbonyl)amino-2-bromo-butanoate (30g), L-leucyl-O-methyl-L-tyrosine N-methylamide (30g) and N-methyl morpholine (9.4g) in acetonitrile (250ml) was stirred and heated under reflux overnight. A further portion of the amine (1.1g) was then added and the solution was heated under reflux for a further 4h. The reaction mixture was then concentrated *in vacuo*, dissolved in chloroform and the solution washed with saturated aq. sodium bicarbonate solution. The material isolated from the organic layer was chromatographed on silica with ethyl acetate as eluant to yield N-[3-N-(Benzyloxycarbonyl)amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide (11.7g); (Found: C,63.09; H,7.46; N,9.59. $C_{30}H_{42}N_{4}O_{7}$ requires C,63.16; H,7.37; N.9.83%); ν_{max} ($CHCl_3$) 3400, 1720 and 1660 cm^{-1}; δ ($CDCl_3$) 0.86

(6H,m,$CH(CH_3)_2$); 1.2-2.1 (6H,m,$NHCH_2CH_2CH$, $CH_2CH(CH_3)_2$,NH); 2.77 (3H,d,J=5Hz,NCH_3); 2.95-3.45 (5H,m,$NHCH_2$, $CH_2C_6H_4$,XCH); 3.66 and 3.76 (each 3H, each s, 2xOCH_3); 3.8 and 4.61 (each 1H, each m, 2x CH); 5.10 (2H,m,$CH_2C_6H_5$); 5.21 (1H,m,OCONH); 6.72 (1H,m,CONH); 6.81 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.12 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.35 (5H,s,C_6H_5); 7.55 (1H,d,J=8Hz,CONH); m/e 571 (100% $[m+1]^+$).

The methyl 4-N-(benzyloxycarbonyl)amino-2-bromobutanoate required in this preparation was made from L-glutamic acid as described in the following paragraphs:

L-Glutamic acid (105g, 0.713 M) was dissolved in concentrated sulphuric acid (300ml) and to this was added chloroform (300ml). To the stirred bi-phasic mixture at 0° was added portionwise over 30 min. sodium azide (60g, 0.9 mole). The reaction mixture was stirred at 5-10° for 30 min. and was then allowed to slowly warm to room temperature. The reaction mixture was then slowly heated to 80° for one hour the reaction was cooled, poured into water (1.5 l) and the aqueous layer was separated. The aqueous extract was diluted (to 20 litres) and was then applied to Dowex 50WX8, 16-40 mesh, H^+ resin. The column was washed with water and then with 1:1 880 Ammonia/Water and the fractions containing the product were lyophilised.

The crude product obtained above was dissolved in

water (1 litre) and to this was added basic copper carbonate (100g). The stirred mixture was heated under reflux for 40 min. and the hot solution was filtered. The solution was cooled to 35° and $NaHCO_3$ (60g) and $CHCl_3$ (300ml) were added. After stirring for 30 min. at room temperature, benzyloxycarbonyl chloride (75ml) was added and the mixture was then allowed to stir at room temperature overnight. A further portion of benzyloxycarbonyl chloride (30ml) was then added and stirring was continued for a further 24h. The crystalline copper complex which had precipitated was then filtered, washed with water and added to a solution of EDTA (di Na salt) (120g) in water (1.5 litre). The resulting mixture was stirred and heated under reflux for 3h and was then cooled to 5°. After 40h at 5° the crystalline product was collected by filtration, washed with water and acetone and dried in vacuo at 45°.

The 4-Z-diamino-butyric acid from above (120g) was suspended in a mixture of dilute sulphuric acid (1M, 600ml), water (200ml) and potassium bromide (240g). Sufficient water (200ml) was then added to form a single phase. To the resulting solution stirred at -7 to -9°, was added a solution of sodium nitrite (44g) in H_2O dropwise over 1h. After 30 min at -7°, the mixture was warmed to room temperature over 1h. Diethyl ether (1.5 litres) was added and the separated aqueous layer was washed with a further portion of ether. The dried

ethereal extracts were concentrated *in vacuo* and the residue in methanol (1 litre) was cooled to 0^{o} and treated dropwise with thionyl chloride (65ml). The reaction was then concentrated *in vacuo* and the residue was partitioned between diethyl ether and saturated aq. sodium bicarbonate. The material isolated from the ether was chromatographed on silica eluting with a gradient of ethyl acetate in hexane to give methyl 4-N-(benzyloxycarbonyl)amino-2-bromo-butanoate (90g) as an oil which crystallised on standing, m.p. 46-50^{o}; (Found: C,47.17; H,5.01; N,4.16. $C_{13}H_{16}BrNO_4$ requires C,47.29; H,4.88; N,4.24%); δ ($CDCl_3$) 2.08-2.45 (2H,m,CH_2); 3.37 (2H,m,$NHCH_2$); 3.76 (3H,s,OCH_3); 4.32 (1H,dd,J=10Hz and 6Hz, CH); 4.97 (1H,broad s, OCONH); 5.09 (2H,s,OCH_2) and 7.34 (5H,s,C_6H_5).

(b) N-[3-N-(Benzyloxycarbonyl)amino-1-(R)-carboxy-propyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a solution of the preceeding ester (171mg, 0.3mM) in methanol (10ml) stirred at 0^{o} was added dilute NaOH (1N, 0.6ml). After stirring overnight at 0^{o} a further portion of NaOH (1N, 0.3ml) was added and the solution was then stirred for 6h at room temperature. The reaction mixture was then acidified with acetic acid and concentrated to a solid *in vacuo*. Recrystallisation of this material from methanol/H_2O gave the *title compound* (150mg); m.p. 170-172^{o}; (Found: C,60.97; H,7.11; N,9.68. $C_{29}H_{40}N_4O_7 \cdot 0.8\ H_2O$ requires C,60.99; H,7.34;

N,9.81%); ν_{max} (Nujol) 3330, 1690 and 1640 cm^{-1}; δ(CD_3OD) 0.88 (6H,dd,J=14Hz and 7Hz, $CH(CH_3)_2$); 1.2-1.95 (5H,m,$NHCH_2CH_2$, $CH_2CH(CH_3)_2$); 2.69 (3H,s,NCH_3); 2.75-3.65 (6H,m,$NHCH_2$, $CH_2C_6H_4$, and α-CHx2); 3.74 (3H,s,OCH_3); 4.54 (1H,dd,J=10Hz and 6Hz, α-CH); 5.08 (2H,m,$CH_2C_6H_5$); 6.82 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.12 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.35 (5H,m,C_6H_5).

Example 12

N-[3-N-(Benzyloxycarbonyl)amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a solution of N-(Tertiarybutoxycarbonyl)-L-leucyl-O-methyl-L-tyrosyl N-methylamide (4.2g, 0.01 M) in CH_2Cl_2 (5ml) at 18° was added trifluoroacetic acid (8ml). After stirring for 2h at room temperature the reaction was concentrated *in vacuo* and was then triturated with dry ether to yield a gum. This was taken up in methanol (25ml), methyl 4-N-(benzyloxycarbonyl)amino-2-oxo-butanoate (4.0g; 0.015 M; *Synthesis*, (1982), 41) was added and the pH of the solution adjusted to 6.5 with triethylamine. To this solution stirred at 0° was added sodium cyanoborohydride (400mg) portionwise whilst the pH was periodically re-adjusted to 6.5 by the addition of acetic acid. After 1h further sodium cyanoborohydride (400mg) was added and the reaction was stirred overnight

at room temperature. After concentration *in vacuo* the residue was partitioned between CH_2Cl_2 (100ml) and water (50ml). The CH_2Cl_2 layer was separated, washed in turn with dilute HCl (1M, 20ml), water (25ml), saturated sodium bicarbonate solution (2x30ml), dried and evaporated to an oil. Chromatography on silica in CH_2Cl_2 in an increasing ethyl acetate gradient gave the *title compound* as a foam (1.0g) which had physical data identical to that given above in Example 11.

Example 13

N-[3-Amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

The acid (320mg, 0.56 mM) from Example 11 in methanol (10ml) was treated with dilute HCl (1M, 1ml). This solution was hydrogenated over 10% palladium on charcoal (60mg) for 90 min. at room temperature, filtered and then concentrated *in vacuo* to give the *title compound* as its dihydrochloride salt; m.p. 149-152^o (from CH_2Cl_2-ether); (Found: C,48.17; H,6.98; N,10.49. $C_{21}H_{34}N_4O_5$.2HCl+0.5 CH_2Cl_2 requires C,48.01; H,6.93; N,10.42%); ν_{max} (Nujol) 3650-2400 (br), 1730 and 1650 cm^{-1}; $\delta(CD_3OD)$ 0.92 and 0.95 (each 3H, each d, each J=15Hz,$CH(C\underline{H}_3)_2$); 1.45-1.90 (3H,m,$C\underline{H}_2C\underline{H}(CH_3)_2$); 2.25 (2H,m,$NHCH_2C\underline{H}_2$); 2.68 (3H,s,$OCH_3$); 3.04 (4H,m,$NHC\underline{H}_2$ and $C\underline{H}_2C_6H_4$); 3.58 (1H,dd,J=8Hz and 6Hz,α-$C\underline{H}$); 3.77 (3H,s,OCH_3); 3.94

(1H,dd,J=8Hz and 4Hz,α-CH); 4.64 (1H,dd,J=13Hz and 6Hz,α-CH); 6.88 (2H,d,J=8.6Hz, Tyr H-3 and H-5) and 7.10 (2H,d,J=8.6Hz, Tyr H-2 and H-6).

Example 14

N-[3-N-(p-Nitrobenzyloxycarbonyl)amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine. N-Methylamide

(a) N-[3-N-(p-Nitrobenzyloxycarbonyl)amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

A solution of N-[3-N-(benzyloxycarbonyl)amino-1-(R)-methoxycarbonyl propyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide (1.24g, mM) in methanol (25ml) containing ethereal HCl (1ml of a 2.6M solution) was hydrogenated over 10% palladised charcoal (0.3g) for 6h at 20°. The solution was filtered and concentrated *in vacuo* to give N-[3-N-amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide dihydrochloride as a foam (1.2g) which was used in the next step without further purification.

To a suspension of N-[3-N-amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide dihydrochloride (400mg, 0.808 mM) in dry CH_2Cl_2 (6ml) cooled in an ice bath, was added p-nitrobenzyloxycarbonyl chloride (400mg) in dry CH_2Cl_2.

To this was then added dropwise a solution of N-methyl morpholine (270mg, 2.67 mM) in dry CH_2Cl_2 (2ml). After 30 min at 0^o, a further portion of p-nitrobenzyloxycarbonyl chloride (400mg) in dry CH_2Cl_2 (1ml) was added followed by a solution of NMM (100mg) in dry CH_2Cl_2 (1ml). After a further 0.5h at 0^o the reaction mixture was diluted with CH_2Cl_2 (20ml), washed in turn with water (20ml), aq. citric acid solution (20ml) and saturated aq. $NaHCO_3$ (20ml). The organic extract was concentrated *in vacuo* and purified by chromatography on silica eluting with CH_2Cl_2 in a rapidly increasing ethyl acetate gradient to give the *title compound* as a foam (450mg, 90%); (Found: $[m+1]^+$=616.3012. $C_{30}H_{42}N_5O_9$ requires $[m+1]^+$=616.2983); $_{max}$ ($CHCl_3$) 3380, 1742 and 1660 cm^{-1}; m/e 616 (5% $[m+1]^+$); 153 (100% $[O_2NC_6H_4CH_2OH]^+$). δ ($CDCl_3$ 0.87 (6H,m,$CH(C\underline{H}_3)_2$); 1.1-2.0 (5H,m,$NHCH_2C\underline{H}_2$, $C\underline{H}_2CH(CH_3)_2$,NH) 2.76 (3H,d,J=5Hz,$NCH_3$); 2.9-3.5 (6H,m,$NHC\underline{H}_2$,$C\underline{H}_2C_6H_4$,$\alpha$-C$\underline{H}$x2) 3.68 and 3.77 (each 3H, each s, 2xOCH_3); 4.60 (1H,dd,J=13Hz and 6Hz,α-C$\underline{H}$);

5.10 (2H,s,$CH_2C_6H_4NO_2$); 5.45 (1H,m,OCONH); 6.50 (1H, broad s, OCONH); 6.82 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.11 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.45 (1H,d,J=8Hz, CONH); 7.52 (2H,d,J=9Hz, benzoyl H-2 and H-6); 8.21 (2H,d,J=9Hz, benzoyl H-3 and H-5).

(b) N-[3-N-(p-Nitrobenzyloxycarbonyl)amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide

To a solution of the preceeding ester (360mg, 0.58mM) in methanol (6ml) at 0^o was added dilute NaOH (1N, 1.2ml). After standing at 0^o for 48h, the solution was acidified with acetic acid and concentrated to a solid *in vacuo*. Trituration with ethyl acetate and water gave the title compound (56mg); m.p. 167-170^o; (Found: C,56.56; H,6.58; N,11.21. $C_{29}H_{39}N_5O_9$+0.8H_2O requires C,56.54; H,6.64; N,11.37%); ν_{max} (Nujol) 3250, 1690 and 1642 cm^{-1}; δ (d^6DMSO) 0.8 (6H,m,$CH(CH_3)_2$; 1.1-2.0 (5H,m,$NHCH_2CH_2$, $CH_2CH(CH_3)_2$); 2.57 (3H,d,J=5Hz,NCH_3); 2.62-3.85 (7H,m,NCH_2, α-CHx2, $CH_2C_6H_4$,OH); 3.67 (3H,s,OCH_3); 4.43 (1H,m,α-CH); 5.10 (2H,s,OCH_2); 6.78 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.13 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.95 (1H,m,CONH); 8.07 (2H,d,J=8.6Hz, Benzoyl H-2 and H-6); 8.25 (1H,m,CONH); 8.31 (2H,d,J=8.6Hz, Benzoyl H-3 and H-5); 9.12 (1H,m,CONH).

Example 15

N-[3-N-(Benzoyl)amino-1-(R)-carboxy-propyl]-L-leucyl-L-tyrosine N-Methylamide

This was prepared in two steps from N-[3-N-amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide as described below:

(a) N-[3-N-(Benzoyl)amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-L-tyrosine N-Methylamide

To a stirred suspension of N-[3-N-amino-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide dihydrochloride (539mg, 1 mM) and benzoyl chloride (186mg, 1mM) in dry CH_2Cl_2 (30ml) at 0^o was added dropwise N-methyl morphline (439mg, 4.3 mM). The reaction mixture was then stirred overnight, concentrated *in vacuo* and chromatographed on silica eluting with ethyl acetate in an ethyl acetate/methanol gradient to yield the *title compound* (350mg); m.p. 145-148^o; (Found: C,63.97; H,7.38; N,10.20. $C_{29}H_{40}N_4O_6$+0.2H_2O requires C,64.00; H,7.48; N,10.29%). $\delta(CDCl_3)$ 0.85 and 0.86 (each 3H, each d, each J=6.5Hz, $CH(C\underline{H}_3)_2$); 1.18-1.80 (4H,m,$NHCH_2C\underline{H}_2CH$ and $CH_2C\underline{H}(CH_3)_2$,NH); 2.0 (2H,dd,J=13 and 6Hz, $C\underline{H}_2CH(CH_3)_2$); 2.75 (3H,d,J=5Hz,$NCH_3$); 3.06 and 3.4-3.7 (6H,m,$NHC\underline{H}_2$, $C\underline{H}_2C_6H_4$ and α-C$\underline{H}$x2); 3.64 and 3.74 (each 3H, each s, 2xOCH_3) 4.60 (1H,dd,J=15Hz and 6Hz, α-C$\underline{H}$); 6.5 and 6.75 (each 1H, each m, 2xCONH); 6.82 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.15 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.5 (5H,m,C_6H_4) and 7.77 (1H,d,J=8Hz,CONH).

(b) N-[3-N-(Benzoyl)amino-1-(R)-carboxypropyl]-L-leucyl-L-tyrosine N-Methylamide

To the preceeding ester (150mg, 0.27 mM) in methanol (10ml) was added dilute NaOH (1N, 1ml) and the solution was then stirred at room temperature for 3 days. The reaction mixture was acidified with acetic acid and was concentrated *in vacuo*. Recrystallisation of the residue from methanol-H_2O gave the title compound (110mg); m.p. 175-177°; (Found: C,61.41; H,7.71; N,10.17. $C_{28}H_{38}N_4O_6$+1.2H_2O requires C,61.34; H,7.34; N,10.22%); ν max (Nujol) 3320 and 1643 cm^{-1}; δ (d^6DMSO) 0.82 (6H,m,$CH(CH_3)_2$); 1.05-2.0 (5H,m,$NHCH_2CH_2$,$CH_2CH(CH_3)_2$); 2.58 (3H,d,J=5Hz,NCH_3); 3.65-4.55 (6H,m,$NHCH_2$),$CH_2C_6H_4$ and α-CHx2); 3.68 (3H,s,OCH_3); 4.42 (1H,m,α-CH); 6.78 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.11 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.46 (3H,m,CONH and 2 protons from C_6H_5); 7.86 (3H,m, 3 protons from C_6H_5); 8.20 (2H,d,J=8Hz,CONH); 8.51 (1H,m,CONH).

Example 16

N-[3-N-(P-Nitrobenzoyl)amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine. N-Methylamide

This was prepared exactly as described for the N-benzoyl derivative in Example 15 except that p-nitrobenzoyl chloride was used in place of benzoyl chloride in the first step. After hydrolysis of the

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intermediate ester, the resulting solid was recrystallised from methanol-water to give the title compound, (450mg); m.p. 170-180°; (Found: C,57.38; H,6.82; N,11.86. $C_{28}H_{37}N_5O_8$+0.8H_2O requires C,57.39; H,6.64; N,11.95%; ν_{max} (Nujol) 3340 and 1645 cm^{-1}; δ(d^6DMSO) 0.82 (6H,m,CH(CH_3)$_2$; 1.05-2.05 (5H,m,NCH_2CH_2CH, $CH_2CH(CH_3)_2$); 2.58 (3H,m,NCH_3); 2.6-3.65 (6H,m,$NHCH_2$$\alpha$-CHx2 and $CH_2C_6H_4$); 3.7 (3H,m,OCH_3); 4.45 (1H,m,α-CH); 6.8 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.12 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.88 (1H,m,CONH); 8.08 (2H,d,J=8Hz, Benzoyl H-2 and H-6); 8.2 (1H,d,J=8Hz,CONH); 8.33 (2H,d,J=8Hz, Benzoyl H-3 and H-5) and 8.88 (1H,m,CONH).

Example 17

N-[3-N-(p-Aminobenzoyl)amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

The acid (351mg), from Example 16 was dissolved in methanol (25ml) and to this solution was added 10% Pd/C (400mg) and dilute ethereal HCl (2.6M, 2ml). after stirring the reaction mixture under hydrogen for 2.5h at room temperature it was filtered and concentrated in vacuo to yield the title compound as a foam (290mg); m.p. 155-160°; (Found: C,50.39; H,6.68; N,10.23. $C_{28}H_{39}N_5O_6$ 3HCl+1H_2O requires C,50.26; H,6.62; N,10.46%); ν_{max} (Nujol) 3650-2120 (broad), 1730 and 1645 cm^{-1}; δ(d^6DMSO) 0.81 and 0.87 (each 3H, each s, CH(CH_3)$_2$); 1.3-1.8 (3H,m,CH(CH_3)$_2$); 2.05

(2H,m,$NHCH_2C\underline{H}_2CH$); 2.58 (3H,d,NCH_3); 2.75 and 2.93 (together 2H, each m, $C\underline{H}_2C_6H_4$); 3.2-3.5 (3H,m,$NHC\underline{H}_2$ and α-C$\underline{H}$); 3.7 (3H,s,OCH_3); 3.97 (1H,m,α-C$\underline{H}$); 4.58 (1H,m,α-C$\underline{H}$); 6.83 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.01 (2H,d,J=8Hz, benzoyl H-3 and H-5); 7.10 (2H,d,J-6.8Hz, Tyr H-2 and H-6); 7.81 (2H,d,J=8Hz, benzoyl H-2 and H-6); 8.17 (1H,m,CONH); 8.67 (1H,m,CONH); 9.11 (1H,d,J=8Hz,CON$\underline{H}$) and 9.5 (3H,br,$N\underline{H}_3$).

Example 18

N-[3-(N'-Benzyl)carbamoyl-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

This was prepared according to the following steps:

(a) N-[3-(N'-Benzyl)carbamoyl-1-(R)-methoxycarbonylpropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a stirred suspension of N-[3-N-amino-1-(R)-methoxy-carbonylpropyl]-L-leucyl-O-methyl-L-tyrosyl N-Methylamide dihydrochloride (406mg, 0.78 mM) in dry CH_2Cl_2 (10ml) at 0° was added benzyl isocyanate (104 μl, 1.56 mM). A solution of N-methyl morpholine (189mg, 1.87 mM) in dry CH_2Cl_2 (5ml) was then added dropwise over 5 min. After 30 min at 0°, a further portion of benzyl isocyanate (25 μl) was added and this was repeated after an additional 30 min. at 0°. The reaction mixture was then allowed to warm to room temperature over 3h. Water (50ml) and CH_2Cl_2 (50ml) were then added and the material isolated from the organic

extracts was chromatographed on silica in 5% MeOH in CH_2Cl_2 to afford the title compound (223mg); 61-69°; (Found: C,62.77; H,7.64; N,12.03. $C_{30}H_{43}N_5O_6$+0.3H_2O requires C,62.65; H,7.64; N,12.18%); δ($CDCl_3$) 0.87 (6H,m,CH($C\underline{H}_3$)$_2$) 1.10-2.0 (6H,m,$NHCH_2C\underline{H}_2$,$C\underline{H}_2C\underline{H}(CH_3)_2$ and NH) 2.64 (3H,d,J=5Hz,$NC\underline{H}_3$); 2.85-3.54 (6H,m,$NHCH_2$, $C\underline{H}_2C_6H_4$ and α-C$\underline{H}$x2); 3.67 and 3.78 (each 3H, each s, 2xOCH_3); 4.37 (2H,dd,15Hz and 2Hz,$C\underline{H}_2C_6H_5$); 4.56 (1H,dd,J=13Hz and 6Hz,α-C$\underline{H}$); 5.16, 5.42 and 6.44 (each 1H, each broad s, 3xCONH) 6.80 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.08 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.3 (5H,m,C_6H_5) and 7.7 (1H,d,J=8Hz,CONH).

(b) N-[3-(N'-Benzyl)carbamoyl-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a solution of the preceeding ester (240mg, 0.42mM) in methanol (25ml) at room temperature, was added dilute NaOH (1N, 1.5ml). After standing overnight at room temperature the reaction mixture was acidified with acetic acid and concentrated in vacuo. Chromatography on reverse phase silica eluting with a methanol/H_2O gradient gave the title compound (107mg); m.p. 104-108°; (Found: C,60.88; H,7.44; N,12.12. $C_{29}H_{41}N_5O_6H_2O$ requires C,60.71; H,7.55; N,12.20%); ν_{max} (Nujol) 3300 and 1640 cm^{-1}; δ(d^6DMSO) 0.8 (6H,m,CH($C\underline{H}_3$)$_2$) 0.95-1.85 (5H,m,$NHCH_2C\underline{H}_2$ and $C\underline{H}_2CH(CH_3)_2$); 2.2-3.4 (6H,m,$NHC\underline{H}_2$, α-C$\underline{H}$x2 and $C\underline{H}_2C_6H_4$); 2.56 (3H,d,J=5Hz, $NHC\underline{H}_3$); 3.70 (3H,s,OCH_3); 4.22 (2H,m,$C\underline{H}_2C_6H_5$); 4.45 (1H,m,α-C$\underline{H}$); 6.0 and 6.42 (each

1H, each m, 2xCONH); 6.82 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.12 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.28 (5H,m,C_6H_5); 7.94 (1H,m,CONH) and 8.25 (1H,d,J=Hz,CONH).

Example 19

N-[3-N-(Benzyloxycarbonyl)amino-1-(R)-carboxypropyl]-L-leucine N-Phenethylamide

N-(Tertiarybutoxycarbonyl)-L-leucine (10g, 0.04M in a mixture of CH_2Cl_2 (100ml) and DMF (10ml) was cooled to 0^o. to this was added 1-hydroxybenzotriazole (6.2g, 0.04 M) followed dropwise by a solution of DCC (8.2g, 0.04 mole) in CH_2Cl_2. After 10 min. at 0^o a solution of phenethylamine (4.84g, 0.04 M) in CH_2Cl_2 (15ml) was added dropwise and the stirred solution was then allowed to warm to room temperature overnight. The reaction mixture was then filtered, concentrated *in vacuo* and dissolved in ethyl acetate (150ml). The ethyl acetate solution was washed in turn with water (40ml), saturated aq. $NaHCO_3$ (50mlx2), aqueous citric acid (50ml) and saturated aq. $NaHCO_3$ (50ml). The residue after evaporation of the solvent was recrystallised from ethyl acetate/hexane to give N-(tertiarybutoxycarbonyl)-L-leucyl-N-phenethylamide as a white powder (9.6g); m.p. 86-88^o; ν_{max} ($CHCl_3$) 3415 and 1670 cm^{-1}; δ ($CDCl_3$) 0.85 (6H,m,$CH(CH_3)_2$); 1.35 (9H,s,$OC(CH_3)_3$); 1.3-1.75 (3H,m,$CH_2CH(CH_3)_2$); 2.69 (2H,t,J=7.2Hz, $CH_2C_6H_5$); 3.3-3.6 (2H,m,NCH_2); 4.05

(1H,m,α-CH); 4.9 (1H,m,OCONH); 6.2 (1H,m,CONH); 7.2-7.4 (5H,m,C_6H_5).

N-(tertiarybutoxycarbonyl)-L-leucine N-phenethylamide (6.17g, mole) was dissolved in a 1:1 TFA/CH_2Cl_2 mixture (60ml). After stirring for 6h at 20° the reaction mixture was concentrated *in vacuo* and the residue in CH_2Cl_2 (50ml) washed with saturated aq. $NaHCO_3$ (100ml). The aqueous extract was back extracted with CH_2Cl_2 (50mlx3) and the combined organic extracts concentrated to an oil *in vacuo*. The crude L-leucine N-phenethylamide so obtained was used as such in the next step.

To a solution of methyl 4-N-(benzyloxycarbonyl)amino-2- bromo-butanoate (330mg, 1 mmole) in dry acetonitrile (10ml) was added L-leucine N-phenethylamide (235mg, 1 mM) and N-methyl morpholine (110mg, 1 mM). The solution was heated at reflux overnight, sodium iodide (150mg, 1mM) was added and the reaction was reheated to reflux for a further 7h. The reaction mixture was then filtered and concentrated to an oil *in vacuo*. Chromatography of the residue on silica in 1:1 EtOAc/Hexane gave N-[3-N-(benzyloxycarbonyl)amino-1-(R,S)-methoxycarbonylpropyl]-L-leucine N-phenethylamide (310mg). Rechromatography on silica then gave the R diastereoisomer as an oil.

To a solution of the foregoing R-isomer (110mg) in methanol (4ml) was added dilute NaOH (1N, 0.5ml).

After standing overnight at 20° the reaction mixture was acidified with acetic acid and concentrated to a solid *in vacuo*. Chromatography on reverse phase silica eluting with 1:1 $MeOH/H_2O$ gave the *title compound* as a white powder (55mg), m.p. 130-135°; (Found: C,65.62; H,7.59; N,8.85. $C_{28}H_{35}N_3O_5+0.3H_2O$ requires C,65.75; H,7.55; N,8.85%); ν_{max} (Nujol) 1690, 1655 and 1630 cm^{-1}; $\delta(d^6DMSO)$ 0.83 (6H,m,$CH(CH_3)_2$); 1.1-1.85 (6H,m,$NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}(CH_3)_2$ and NH); 2.69 (2H,t,J=7.2Hz,$C\underline{H}_2C_6H_5$); 3.0-3.6 (7H,NCH_2x2,α-C$\underline{H}$x2, CO_2H); 5.0 (2H,s,$OC\underline{H}_2C_6H_5$); 7.1-7.5 (10H,m,C_6H_5x2); 8.05 (1H,m,CONH).

Example 20

N-[5-N-(Benzyloxycarbonyl)amino-1-(R)-methoxycarbonyl pentyl)-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a stirred solution of crude methyl 6-N-(benzyloxycarbonyl)amino-2-oxo-hexanoate (7.03g, 24mM; *Tet.Lett.*, (1982), 23, 1875) and L-leucyl-O-methyl-L-tyrosine N-Methylamide (1.86g, 6 mM) in methanol (50ml) was added acetic acid to bring the pH to 6.5. Sodium cyanoborohydride (400mg, 6.5mM) was then added portionwise whilst the pH of the solution was continually re-adjusted to 6.5 by the addition of acetic acid. After 1.5h at room temperature a further portion of sodium cyanoborohydride (400mg) was added and the pH was again re-adjusted to 6.5 with acetic acid. After a further 1h at room temperature, the reaction mixture was

concentrated *in vacuo* and the residue in CH_2Cl_2 (50ml) was washed in turn with water (30ml), dilute HCl (1M, 30ml) and saturated aq.$NaHCO_3$. The material isolated from the organic layer was purified by column chromatography on silica in CH_2Cl_2 in an increasing ethyl acetate gradient to give the title compound as an oil (360mg); (Found: $[m+1]^+$= xxx.xxxx. $C_{32}H_{44}N_4O_7$ requires $[m+1]^+$= xx.xxxx); δ ($CDCl_3$) 0.88 $CH(C\underline{H}_3)_2$); 1.0-1.86 (10H,m,$NHCH(C\underline{H}_2)_3$), $C\underline{H}_2C\underline{H}(CH_3)_2$ and NH); 2.74 (3H,d,J=5Hz,NCH_3); 2.85-3.4 (6H,m,$NHC\underline{H}_2$,$C\underline{H}_2C_6H_4$ and α-C$\underline{H}$x2); 3.65 and 3.75 (each 3H, each s, 2xOCH_3); 4.64 (1H,dd,J=13Hz and 6Hz, α-C$\underline{H}$); 5.10 (2H,s,$C\underline{H}_2C_6H_5$); 6.78 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.10 (2H,d,J=8.6Hz, Tyr H-2 and H-6); 7.35 (5H,m,C_6H_5) and 7.64 (1H,d,J=10Hz,CONH).

Example 21

N-[5-N-(Benzyloxycarbonyl)amino-1-(R)-carboxypentyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

To a solution of the ester from Example 20 (140mg, 0.23 mM) in methanol (10ml) at 0^o was added dilute NaOH (1N, 0.5ml). After 48h at 0^o, a further portion of NaOH (1N, 0.4ml) was added and the solution stirred at 20^o for a further 24h. The reaction mixture was then acidified with acetic acid and concentrated *in vacuo* to give a semi-solid which was purified by partition between ethyl acetate and water at 0^o. The resulting solid was filtered, washed with water and ethyl acetate

and was dried *in vacuo* to give the title compound (110mg); 122-128°; (Found: $[m+1]^+$=585.3290 $C_{31}H_{44}N_4O_7$ requires $[m+1]^+$=585.3288) ν_{max} (Nujol) 3340, 1688 and 1640 cm^{-1}; $\delta(CD_3OD)$ 0.88 (6H,m,$CH(CH_3)_2$); 1.0-1.86 (9H,m,$NHCH_2(CH_2)_3$ and $C\underline{H}_2C\underline{H}(CH_3)_2$); 2.74 (3H,s,$NCH_3$); 2.8-3.6 (6H,m,$NHCH_2$,$CH_2C_6H_4$ and α-$C\underline{H}$x2); 3.77 (3H,s,OCH_3); 4.60 (1H,m,α-$C\underline{H}$); 5.10 (2H,s,$CH_2C_6H_5$); 6.78 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.05 (1H,m,CONH); 7.10 (2H,d,J=8.6Hz Tyr H-2 and H-6) and 7.35 (5H,m,C_6H_5); m/e 585 (1%, $[m+1]^+$), 567 (20% $[m+1-H_2O]^+$).

Example 22

N-[5-N-[N-Acetyl-L-prolyl]amino-1-(R)-carboxypentyl]-L-leucyl-O-methyl-L-tyrosine N-Methylamide

N-[5-N-(Benzyloxycarbonyl)amino-1-(R)-methoxycarbonyl-pentyl)-L-leucyl-O-methyl-L-tyrosine N-methylamide (400mg, 0.66 mM) in methanol (20ml) was treated with dilute HCl (1N, 1.2ml) and $PdCl_2$ (50mg). The reaction mixture was stirred under hydrogen for 20 min. at room temperature and was then filtered. Concentration of the resulting solution *in vacuo* gave N-[5-amino-1-(R)-methoxycarbonylpentyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide hydrochloride as an oil. This was dissolved in CH_2Cl_2 (20ml) and DMF (5ml) and to the resulting solution was added N-methyl morpholine (300mg) and N-acetyl-L-proline p-nitrophenyl

ester (191mg). After standing at 20° for 72h, the reaction mixture was concentrated *in vacuo* and the residue in ethyl acetate (20ml) was washed with aq. citric acid solution. These aqueous washings were concentrated *in vacuo* and the resultant oil was purified by chromatography on reverse phase silica eluting with a gradient of methanol in H_2O to give N-[5-N-(N-acetyl-L-prolyl)amino-1-(R)-methoxycarbonylpentyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide (350mg) $\delta(CDCl_3)$ 0.84 (6H,dd,J=14Hz and 7Hz,$CH(C\underline{H}_3)_2$); 1.05-2.4 (13H,m,$NHCH_2(CH_2)_3$, $C\underline{H}_2C\underline{H}(CH_3)_2$ and CH_2CH_2); 2.08 (3H,s,$COCH_3$); 2.70 (3H,s,NCH_3); 2.76-3.82 (8H,m,NCH_2,$NHC\underline{H}_2C_6H_4$ and α-CHx2); 3.66 and 3.74 (each 3H, each s, $2xOCH_3$); 4.32 (1H,m,α-C$\underline{H}$); 4.56 (1H,dd,J=13Hz and 6Hz,α-C$\underline{H}$); 6.80 (2H,d,J=8.6Hz, tyr H-3 and H-5) and 7.12 (2H,d,J=8.6Hz, Tyr H-2 and H-6).]

A portion of this material (130mg) in methanol (5ml) was treated at 0° with dilute NaOH (1N, 0.5ml). After standing overnight at room temperature, a further portion of NaOH was added (1N, 0.2ml) and this was then repeated 6h later. After a further 18h at 20° the reaction mixture was acidified with acetic acid and concentrated to an oil *in vacuo*. Chromatography on reverse phase silica eluting with water in an increasing methanol gradient gave the *title compound* (100mg); m.p. 97-101°; (Found: $[m+1]^+$=590.3552 $C_{30}H_{47}N_5O_7$ requires $[m+1]^+$=590.3554); ν_{max} (Nujol 3280 (br) and 1625 (br) cm^{-1}; $\delta(CD_3OD)$ 0.94 (6H,m,$CH(C\underline{H}_3)_2$); 1.2-2.4

(13H,m,$NHCH_2(CH_2)_3$, $CH_2CH(CH_3)_2$ and CH_2CH_2); 2.12 (3H,s,$COCH_3$); 2.68 (3H,s,NCH_3); 2.75-4.1 (8H,m,NCH_2,$NHCH_2$,$CH_2C_6H_4$ and α-CHx2); 3.77 (3H,s,OCH_3); 4.33 and 4.58 (each 1H, each m, 2x2CH); 6.85 (2H,d,J=8.6Hz, Tyr H-3 and H-5); 7.16 (2H,d,J=8.6Hz, Tyr H-2 and H-6) and 8.03 (1H,m,CONH); m/e 590 (2%, $[m+1]^+$, 572 (10% $[m+1-H_2O^+]$).

Example 23

N-[2-(S)-N-(1-(R)-Carboxyethyl)amino-4,4-dimethylpentanoyl] -L-alanine N-Butylamide

N-[2-(S)-N(1-(R)-Methoxycarbonylethyl)amino-4,4-dimethylpentanoyl]-L-alanine N-butylamide (65mg) in methanol (30ml) was treated with lN-sodium hydroxide (3ml) at 20° for 6h. Excess acetic acid was then added and the solvent evaporated *in vacuo*. The residue was chromatographed on reverse phase silica (RP 18) in a gradient of 20%-80% methanol in water. Elution in 70% methanol in water afforded the title compound (30mg) as a freeze-dried powder, m.p. 137-138°; (Found: $[m+1]^+$=344.2548. $C_{17}H_{34}N_3O_4$ requires $[m+1]^+$=344.2549); δ(D_2O) 0.9 (3H,t,J=6Hz,CH_2CH_3); 0.94 (9H,s,$C(CH_3)_3$); 1.2-1.8 (6H,m,$(CH_2)_2$ and CH_2); 1.4 (3H,d,J=8Hz,CH_3); 1.52 (3H,d,J=7Hz,CH_3); 3.18 (2H,t,J=6Hz,$NHCH_2$); 3.66 (1H,q,J=5Hz,CHCO); 3.88 (1H,d,J=10Hz,$CHCH_2$) and 4.38 (1H,q,J=5Hz,$CHCH_3$).

The starting material required in the preceeding preparation was synthesised as described in the

following paragraphs:

(a) Benzyl 2-Bromo-4,4-dimethylpentanoate

4,4-Dimethylpentanoic acid (40g; Chem Lett, (1980), 571) was treated at 20° for 16h with thionyl chloride (40g) and the mixture distilled under reduced pressure to yield 4,4-dimethylpentanoyl chloride (38g) b.p. 52-58° at 10mm Hg; δ ($CDCl_3$) 0.94 (9H,s,$C(CH_3)_3$; 1.66 (2H,t,J=9Hz,CH_2) and 2.88 (2H,t,J=9Hz,CH_2CO) .

A portion of this material (20g) was treated at 110° with bromine (20g) for 4h. Further bromine (5g) was then added and the reaction continued for 1h. Distillation under reduced pressure afforded 2-bromo-4,4-dimethylpentanoyl chloride (26g), b.p. 92-96° at 10mmHg; δ ($CDCl_3$) 1.0 (9H,s,$C(CH_3)_3$); 1.94 (1H,dd,J=15 and 5Hz, C$\underline{H}$CHBr); 2.42 (1H,dd,J=15 and 8Hz, C$\underline{H}$CHBr) and 4.64 (1H,dd,J=8 and 5Hz,C$\underline{H}$Br) .

The bromo-acid chloride (12g) in CH_2Cl_2 (100ml) was treated with benzyl alcohol (8.8g) and N-methyl morpholine (4.06g) at 0° for 16h. The solution was then washed successively with dilute HCl and Sat.aq.$NaHCO_3$ solution. The residue after evaporation of the solvent was purified by chromatography on silica in 20% ether-hexane to give the desired bromo ester (11.2g) as an oil; (Found: C,56.3; H,6.4; Br,26.8; $C_{14}H_{19}Br,O$ requires C,56.2; H,6.4; Br,26.7%); ν_{max} 2940 and 1730 cm^{-1} δ($CDCl_3$) 0.88 (9H,s,$(CH_3)_3C$); 1.92 (1H,dd,J=15 and 4Hz,C$\underline{H}$CHBr); 2.38 (1H,dd,J=15 and 10Hz,C$\underline{H}$CHBr); 4.34 (1H,dd,J=10 and 4Hz C$\underline{H}$Br); 5.2

($2H,s,OC\underline{H}_2-C_6H_5$) and 7.4 ($5H,m,C_6H_5$).

(b) Benzyl 2-(S)-N-(1-(R)-Methoxycarbonylethyl)amino-4,4-dimethylpentanoate

Benzyl-2-bromo-4,4-dimethylpentanoate (20g) in dry dimethyl sulphoxide (250ml) was treated with D-alanine methylester hydrochloride (9.33g), N-methyl morpholine (6.78g) and tetrabutyl ammonium iodide (24.7g) at 90° under an atmosphere of argon for 2 days. The reaction mixture was allowed to cool to room temperature, poured into water (500ml) and the products recovered by extraction into dichloromethane (3x250ml). The material isolated from the organic extracts was purified by chromatography on silica developed in a gradient of hexane-ether. Elution with 30% ether-hexane afforded benzyl 4,4-dimethylpent-2-enoate (14g). Elution with 40% ether in hexane afforded the title compound (350mg) as a gum; (Found: $[m+1]^+=322.2022$. $C_{18}H_{27}N_1O_4$ requires $[m+1]^+=322.2016$); ν_{max} (film) 1735 cm^{-1}; $\delta(CDCl_3)$ 0.90 ($9H,s,C(CH_3)_3$); 1.28 ($3H,d,J=7Hz\ CHC\underline{H}_3$); 2.46 and 2.68 ($2H$, each dd,$J=12$ and $5Hz,CH_2(CH_3)_3$); 3.30 ($1H,q,J=5Hz\ C\underline{H}-CH_3$); 3.36 ($1H,t,J=5Hz,\ C\underline{H}-CH_2$); 3.66 ($3H,s,OCH_3$); 5.12 ($2H,s,OCH_2$) and 7.36 ($5H,s,C_6H_5$). Elution with 45% ether in hexane afforded benzyl 2-(R)-N-(1-(R)-methoxycarbonylethyl)-amino-4,4-dimethylpentanoate (340mg); (Found: $[m+1]^+=322.2022$. $C_{11}H_{27}NO_4$ requires 322.2016); ν_{max} (film) 3360 and 1735 cms^{-1}; $\delta(CDCl_3)$ 0.90 ($9H,s,C(CH_3)_3$); 1.28 ($3H,d,J=6Hz,\ CHC\underline{H}_3$); 1.44 and 1.72 (2H,

each dd, J=5 and 12.5Hz, CH_2); 3.32 (1H, q, J=7Hz, C<u>H</u>CH_3); 3.44 (1H, t, J=6Hz, C<u>H</u>CH_2); 3.69 (3H, s, OCH_3), 5.24 (2H, s, OCH_2) and 7.36 (5H, m, C_6H_5).

(c) <u>N-[2-(S)-N(1-(R)-Methoxycarbonylethyl)amino-4,4-dimethylpentanoyl]-L-alanine N-Butylamide</u>

The foregoing benzyl ester (450mg) in methanol (50ml) was treated with palladium on charcoal (10% 400mg) under 1 atmosphere of hydrogen with continuous stirring. When the uptake of hydrogen had ceased (15 min) the solution was filtered and the filtrate concentrated <u>in vacuo</u> to afford 2-(S)-N-(1-(R)-methoxycarbonylethyl)amino-4,4-dimethylpentanoic acid (210mg); m.p. 120-124° (from ether).

This material (200mg) in CH_2Cl_2 (50ml) was treated with L-alanine N-butylamide hydrochloride (220mg), N-ethyl-N'-(3-dimethylamino propyl) carbodiimide hydrochloride (200mg) and 1-hydroxybenzotriazole (120mg) at 0°C. The pH of the reaction mixture was adjusted to 7 by the addition of N-methyl morpholine. After 16h at 20°, the solution was washed in turn with saturated sodium hydrogen carbonate solution and 1M citric acid solution. The material isolated after evaporation of the dichloromethane was chromatographed on silica developed in a gradient of 20% ethyl acetate in dichloromethane to 60% ethyl acetate in dichloromethane to afford the <u>title compound</u> (110mg) as a colourless oil, (Found: $[m+1]^+$=358.2705. $C_{18}H_{35}N_3O_4$ requires $[m+1]^+$=358.2706);

($CDCl_3$) 0.92 (3H,t,J=7.5Hz,$CH_2C\underline{H}_3$); 1.0 (9H,s,$C(CH_3)_3$); 1.36 and 1.40 (each 3H, each t, J=6Hz,$2xCH_3$); 1.2-1.9 (6H,m,$3xCH_2$); 3.24 (2H,m,$NHC\underline{H}_2$); 3.46 (1H,q,J=6Hz,C$\underline{H}$); 3.77 (3H,s,OCH_3), 4.46 (1H,t,J=6Hz,$C\underline{H}CH_2$); 4.5 (1H,q,J=6Hz,C$\underline{H}$), 7.15 (1H,m,NH) and 7.73 (1H,d,J=8Hz,NH).

The L-alanine N-butylamide hydrochloride used in step (c) was prepared from N-tertiarybutoxycarbonyl-L-alanine N-butylamide by exposure to TFA in CH_2Cl_2 followed by treatment with ethereal HCl. This in turn was prepared from N-tertiarybutoxy-L-alanine and n-butylamine following the procedure described in Example 2 for N-tertiarybutoxy-O-benzyl-L-tyrosine N-methylamide except that butylamine was used in place of methylamine hydrochloride.

Example 24

N-(1-(R)-Carboxyethyl)-S-norleucyl-S-alanine, N-Butylamide

This was prepared from tertiarybutoxycarbonyl-L-norleucine, L-alanine N-butylamide and 2-bromopropionic acid methyl ester as described in the following steps:

(a) Tertiarybutoxycarbonyl-L-norleucyl-L-alanine N-butylamide

Tertiarybutoxycarbonyl-L-norleucine (13.2g) in CH_2Cl_2 (200ml) was treated at 0^o with L-alanine N-butylamide (5.25g), DCC (7.77g) and

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1-hydroxybenzotriazole (5g). The pH of the reaction mixture was adjusted to 7 with N-methyl morpholine and allowed to warm to room temperature overnight. The precipitated urea was filtered off and the filtrate washed successively with saturated aqueous sodium hydrogen carbonate, water and 1M citric acid. The organic phase was dried over sodium sulphate and the solvent evaporated *in vacuo*. The residue was chromatographed on silica in a gradient of 30-70% ethyl acetate in dichloromethane. Elution with 50% ethyl acetate in dichloromethane afforded the *title compound* (7.6g) which crystallised from ethyl acetate as needles m.p. 108-112^{o}; (Found: C,60.8; H,9.8; N,11.8. $C_{18}H_{35}N_3O_4$ requires C,60.5; H,9.9; N,11.75%); ν_{max} (Nujol) 3280, 3340 1675 and 1640 cms^{-1} δ ($CDCl_3$) 0.9 and 0.91 (each 3H, each t, each J=5Hz,2xCH_3); 1.1-1.9 (10H,m,$(CH_2)_3$ and $(CH_2)_2$); 1.38 (3H,d,J=5Hz,6H_2 $CHC\underline{H}_3$); 1.44 (9H,s, $C(CH_3)_3$); 3.24 (2H,tt,J=5Hz $NHC\underline{H}_2$) and 4.1 and 4.48 (each 1H, each m, 2x $C\underline{H}$).

(b) L-Norleucine-L-alanine N-butylamide

Tertiarybutonycarbonyl-L-norleucine-L-alanine N-butylamide (5g) in dichloromethane (20ml) was treated with trifluroacetic acid (20ml) at room temperature for 2h. The solvents were evaporated *in vacuo* and the residue in water was treated with excess sodium hydrogen carbonate and the free amine recovered in dichloromethane. Evaporation of the CH_2Cl_2 and crystallisation of the residue from ether-hexane gave

the *title compound* (3.1g); m.p. 83-84°; (Found: C,60.7; H,10.4; N,16.0. $C_{13}H_{27}N_3O_2$ requires C,60.6; H,10.6; N,16.3%); ν_{max} (Nujol): 3360, 3280, 1635 and 1675 cm^{-1}; $\delta(CDCl_3)$ 0.94 (6H,t,J=5Hz,$2xCH_2C\underline{H}_3$); 1.40 (3H,d,J=6Hz $CH-C\underline{H}_3$); 1.4-1.9 (10H,m,$(CH_2)_3$ and $(CH_2)_2$); 3.26 (2H,dt, each J=5Hz,$NH-C\underline{H}_2-$); 3.35 (1H,dd,J=4 and 8Hz, $C\underline{H}-CH_2$); 4.50 (1H,dq, each J=6Hz, $C\underline{H}-CH_3$); 6.9 (1H,m,NH); 7.86 (1H,d,J=7Hz,N$\underline{H}$).

(c) N-(1-(R)-Methoxycarbonylethyl)-S-norleucyl-S-alanine N-Butylamide

L-Norleucine-L-alanine N-butylamide (1g) in acetonitrile (10ml) was treated with N-methyl morpholine (0.4g) and methyl 2-bromopropionate (0.64g) under reflux for 16h. The solvent was removed *in vacuo* and the residue in dichloromethane washed successively with 1M citric acid, water and saturated aqueous sodium hydrogen carbonate. The residue after evaporation of the CH_2Cl_2 was chromatographed on silica in a gradient of ethyl acetate in CH_2Cl_2. Elution with 60% ethyl acetate in CH_2Cl_2 afforded N-(1-(S)-methoxy-carbonylethyl)-S-norleucyl-S-alanine N-butylamide (210mg); (Found: $[m+1]^+$=344.2547. $C_{17}H_{34}N_3O_4$ requires $[m+1]^+$=344.2582); ν_{max} (Nujol) 3320 and 1740 cms^{-1}; $\delta(CDCl_3)$ 0.95 (6H,t,J=7Hz,$2xCH_2C\underline{H}_3$); 1.36 and 1.40 (each 3H, each d, each J=6Hz,$2xCHC\underline{H}_3$); 1.2-1.8 (10H,m,$(CH_2)_2$ and $(CH_2)_3$); 2.98 (1H,dd,J=4 and 5Hz, $C\underline{H}CH_2$); 3.24 (3H,m,$NHC\underline{H}_2$ and CHCO); 3.7 (3H,s,OCH_3); 4.56 (1H,dq,J=5Hz,CH) and 7.04 and 7.9 (each 1H, each m,

2xNH).

Continued elution with 65% ethyl acetate in CH_2Cl_2 gave the title compound (190mg), m.p. 84-88° (from ethyl acetate); (Found: C,59.2; H,9.5; N,12.2. $C_{17}H_{33}N_3O_4$ requires C,59.6; H,9.4; N,12.3%); ν_{max} (Nujol) 3280 and 1740 cms^{-1}; δ ($CDCl_3$) 0.94 (6H,t,J=6Hz,2xCH_2CH_2); 1.38 and 1.42 (each 3H, each d, each J=5Hz, 2x$CHCH_3$); 1.3-1.9 (10H,m, $(CH_2)_2$); 3.06 (1H,dd,J=5 and 8Hz, $CHCH_2$); 3.24 (2H,dt,J=5 and 6Hz,$NHCH_2$); 3.46 (1H,q,J=6Hz,CHCO); 3.72 (3H,s,OCH_3); 4.67 (1H,dq,J=5 and 7Hz,$CHCH_3$); 6.84 (1H,m,NH) and 7.82 (1H,d,J=7Hz,NH).

(d) N-(1-(R)-Carboxyethyl)-S-norleucyl-S-alanine, N-Butylamide

The foregoing methyl ester (150mg) in CH_3OH (50ml) was treated with 1M NaOH (1ml) at room temperature for 72h. Excess acetic acid was added and the solvents evaporated *in vacuo*. The residue was chromatographed on reverse phase silica (RP18) in a gradient of 0-60% methanol in water. Elution with 50% methanol in water afforded the title compound (110mg) as needles from ether/hexane; m.p. 185-190°; (Found: C,56.7; H,9.2; N,12.4. $C_{16}H_{31}N_3O_4.H_2O$ requires C,56.8; H,9.5; N,12.4%); ν_{max} (Nujol) 3200 and 1650 cm^{-1}; δ (CD_3OD) 0.92 and 0.94 (each 3H, each t, each J=6Hz,2xCH_2CH_3); 1.36 and 1.48 (each 3H, each d, each J=6Hz,2x$CHCH_3$); 1.2-1.9 (10H,m,$(CH_2)_2$ and $(CH_2)_3$); 3.20 (2H,t,J=6Hz $NH-CH_2$); 3.56 (1H,q,J=6Hz,$CHCO_2H$); 3.86

The compounds of Examples 25 to 131 and their routes of preparation are exemplified within the following Tables.

Using the methods illustrated in examples 1-24 further examples 25-131 in Table 1 are prepared.

Compounds N-[2-(S)-N-(1-(R)-carboxyethyl) amino-4,4-di-(trifluoromethyl)butanoyl]-O-methyl-L-tyrosine N-methylamide and N-[2-(S)-N-(3-(benzyloxycarbonyl) amino-1-(R)-carboxypropyl)amino-4,4-di-(trifluoromethyl) butanoyl]-O-methyl-L-tyrosine N-methylamide are likewise prepared by methods described in examples 1-24.

TABLE 1

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREOCHEM[1]	MP. °C[2] R^1=OCH_3	MP. °C[2] R^1=OH
25	1A	-	H	-	1	H	$CH(CH_3)CH_2CH_3$	$GlyNHC_4H_9^n$	RS	82-84	74-77
26	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$GlyNHC_4H_9^n$	RS		87-95
27	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$GlyNHC_4H_9^n$	SS		175-180
28	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$ValNHC_6H_{13}^n$	RSS		190-193
29	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$ValNHC_6H_{13}^n$	SSS		200-203
30	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$LeuNHC_4H_9^n$	RSS	138-139	180-185
31	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$LeuNHC_4H_9^n$	SSS	180-185	183-185
32	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$LeuNHC_4H_9^n$	RSR	103-107	150-160
33	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$LeuNHC_4H_9^n$	SSR	94-98	185-188
34	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$Thr(OBZ)NHC_4H_9^n$	RSSR	62-67	145-148
35	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$Thr(OBZ)NHC_4H_9^n$	SSSR	61-64	147-152
36	1A	-	H	-	3	H	$CH_2CH(CH_3)_2$	$ValGlyOCH_3$	RS		87-92
37	1A	-	H	-	3	H	$CH_2CH(CH_3)_2$	$ValGlyOCH_3$	SS		177-180
38	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$ValGlyOCH_3$	RS		

TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREO-CHEM[1]	MP. °C[2] R^1=OCH_3	MP. °C[2] R^1=OH
39	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RSSR	72-76	194-197
40	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NH(CH_2)_2$-SCH_2CH_3	RSSR	162-164[3]	105-109
41	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NH(CH_2)_2$-$SOCH_2CH_3$	RSSR		80-85
42	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NH(CH_2)_3$-$CONH_2$	RSSR	97	193
43	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NH(CH_2)_5$-$CONH_2$	RSSR	foam	115-120
44	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$N(CH_3)$-$C_4H_9{}^n$	RSSR	oil	56-57
45	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NH(CH_2)_2$-$SO_2CH_2CH_3$	RSSR	157-161[3]	90-95
46	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NH(CH_2)_5$-CO_2H	RSSR	131-133[3]	105-107

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TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREOCHEM[1]	MP. °C[2] $R^1=OCH_3$	MP. °C[2] $R^1=OH$
47	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$NHCH(CONHCH_3)CH_2$-$NHCO_2C(CH_3)_3$	RSS	107-112	174-182
48	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Ser(OBZ)$NHCH_3$	RSS	61-64	188-190
49	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Tyr$NHCH_3$	RSS		212-217
50	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$NHCH(CONHCH_3)CH_2$-NHCOPh	RSS	71-74	186-191
51	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$NHCH(CONHCH_3)CH_2$-NHZ	RSS	110-112	163-166
52	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Thr$NH(CH_2)_3CONH_2$	RSSR		235
53	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Ala$NHC_4H_9{}^n$	RSS	174-176[3]	158-162
54	1A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	Ala$NHCH_3$	SSS		176-182
55	1A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	Ala$NHCH_3$	RSS		166-168
56	1A	-	H	-	6	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	SSSR	62-64	112-120
57	1A	-	H	-	6	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RSSR	79-80	63-66
58	1A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RSSR	92-94	160-164
59	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Ala$NHCH_3$	RSS	87-90	84-88

TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREO-CHEM[1]	MP. °C[2] $R^1=OCH_3$	MP. °C[2] $R^1=OH$
60	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$PheNHCH_3$	RSS	116-119	115-116
61	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$SarNHCH_3$	RS	160-175	77-80
62	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$ProNHCH_3$	RSS	99-102	100-105
63	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$NHCH(CONHCH_3)-(CH_2)_6CH_3$	RS(RS)	155-159[3]	186-191
64	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$N-CH_3-Tyr(OBZ)-NHCH_3$	RSS	115-120[3]	115-121
65	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$iso-AbaNHCH_3$	RS	150-153[3]	177-179
66	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$N-Z-LysNHCH_3$	RSS	170-172[3]	162-164
67	1A	Z	Leu	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	SRSS		145-150
68	1A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$SerNHCH_3$	RSS		191-198
69	1A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	$AlaNHCH_3$	SSS		176-18[illegible]
70	1A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	$AlaNHCH_3$	RSS		166-16[illegible]

TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREO-CHEM[1]	MP. °C[2] $R^1=OCH_3$	MP. °C[2] $R^1=OH$
71	1B	-	H	NH	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RSSR		105-107
72	1B	-	CH_3CO	NH	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RSSR	131-132	108-112
73	1B	-	CH_3CO	NH	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RRSR	133-134	100-102
74	1B	-	$(CH_3)_3COCO$	NH	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RSSR	95-97	114-118
75	1B	-	$(CH_3)_3COCO$	NH	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	RRSR	123-124	80-90
76	1B	DnpPro	Leu	NH	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	SSRSSR		112-115
77	1B	DnpPro	Leu	NH	1	H	$CH_2CH(CH_3)_2$	Thr(OBZ)$NHCH_3$	SSRRSR		108-115
78	1B	-	$Ph(CH_2)_2NHCO$	-	1	H	$CH_2CH(CH_3)_2$	Tyr(OCH_3)$NHCH_3$	R(RS)S	124-128	
79	1B	-	HO_2C	-	1	H	$CH_2CH(CH_3)_2$	Tyr(OCH_3)$NHCH_3$	RSS	64-66	130-132
80	2A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Tyr(OBZ)NH_2	RSS	117-119[4]	193-196
81	2A	-	H	-	1	H	$CH_2CH(CH_3)_2$	Tyr(OCH_3)$NHCH_3$	RSS	96-97[4]	200-202
82	2A	-	H	-	1	H	$CH(CH_3)_2$	$AlaNHC_4H_9^n$	SSS	60-62	100-10[illegible]
83	2A	-	H	-	1	H	$CH(CH_3)_2$	$AlaNHC_4H_9^n$	RSS		165-17[illegible]
84	2A	-	H	-	1	H	CH_3	$AlaNHC_4H_9^n$	(RS)SS		220-22[illegible]
85	2A	-	H	-	1	H	CH_3	$AlaNHC_4H_9^n$	(SR)SS		231-234
86	2A	-	H	-	1	H	$CH_2CH(CH_3)_2$	His(BZ)$NHCH_3$	RSS		95-103

TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREO-CHEM	MP. °C R^1=OCH_3	MP. °C R^1=OH
87	2A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$AlaNHOCH_3$	RSS		192-196
88	2A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$Thr(OC_4H_9{}^t)NHCH_3$	RSSR		98-108
89	2A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$TyrNH_2$	RSS		219-230
90	2A	-	H	-	1	H	$(CH_2)_2CH_3$	$AlaNHC_4H_9{}^n$	R(RS)S	84-85	199-201
91	2A	-	CH_3CO	NH	3	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS	101-104	150
92	2A	-	H	-	1	H	$(CH_2)_2SCH_3$	$AlaNHC_4H_9{}^n$	RSS	foam	155-159
93	2A	-	H	-	1	H	$CH_2CH(CH_3)_2$	$NHCH(CH_3)CH_2Ph$	RS(RS)		
94	2A	-	H	-	1	H	$CH_2C_6H_5$	$AlaNHC_4H_9{}^n$	(RS)SS		173-178
95	2A	-	H	-	1	H	CH_2OCH_2Ph	$AlaNHC_4H_9{}^n$	RSS		158-162
96	2A	-	H	-	1	H	$CH(CH_3)CH_2CH_3$	$AlaNHC_4H_9{}^n$	RSS	90-94	162-164
97	2A	-	$Ph(CH_2)_2CO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS	114-118	162-163
98	2A	-	H	-	1	$-(CH_2)_5-$		$AlaNHC_4H_9{}^n$	(RS)S		95-105
99	2A	-	$(CH_3)_2CHCH_2CO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		168-172
100	2A	-	CH_3OCO	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		149-153
101	2A	Z	Pro	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	SRSS	102-103	174-179
102	2A	-	$(CH_3)_2CHCH_2OCO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		171-175

TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREO-CHEM	MP. °C $R^1=OCH_3$	MP. °C $R^1=OH$
103	2A	-	PhCH=CHCO	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		188-191
104	2A	-	$2\text{-}Cl\text{-}C_6H_4CO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS	109	158-163
105	2A	-	$4\text{-}Cl\text{-}C_6H_4CO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS	105-108	187-192
106	2A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)OC_4H_9{}^t$	RSS		101-102
107	2A	-	$4\text{-}CH_3\text{-}C_6H_4CH_2\text{-}OCO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS	141-143	164-167
108	2A	-	$4\text{-}Cl\text{-}C_6H_4CH_2OCO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		167-171
109	2A	-	$HO_2C(CH_2)_2CO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS	50-55	160-171
110	2A	-	$4\text{-}CH_3\text{-}C_6H_4CO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS	98	190-194
111	2A	-	$PhCH_2CO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		173-179
112	2A	-	$2\text{-}Cl\text{-}C_6H_4CH_2OCO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		168-17[illegible]
113	2A	-	$4\text{-}CH_3O\text{-}C_6H_4CH_2\text{-}OCO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		162-16[illegible]
114	2A	-	Bornyl-OCO	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		145-15[illegible]
115	2A	-	$2\text{-}CH_3\text{-}C_6H_4CH_2\text{-}OCO$	NH	2	H	$CH_2CH(CH_3)_2$	$Tyr(OCH_3)NHCH_3$	RSS		170-173

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TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREO-CHEM	MP. °C R^1=OCH$_3$	MP. °C R^1=OH
116	2A	-	Ph$(CH_2)_2$OCO	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH3)NHCH$_3$	RSS		147-151
117	2A	-	PhCH$_2$SO$_2$	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS	50-60	174-178
118	2A	-	PhCH$_2$N(CH$_3$)CO	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS	65-70	90-95
119	2A	-	2-NaphthylCO	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS	148-153	162-172
120	2A	-	1-NaphthylCO	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS	67-71	167-173
121	2A	-	Ph	-	1	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS		161-166
122	2A	-	1-NaphthylCH$_2$-OCO	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS	85-86	182-184
123	2A	-	2-NaphthylCH$_2$-OCO	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS	92-98	168-171
124	2A	-	PhC≡CCO	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS		
125	2A	-	H	-	1	H	$CH_2CH(CF_3)_2$	Tyr(OCH$_3$)NHCH$_3$	RSS		
126	2A	-	Z	NH	2	H	$CH_2CH(CF_3)_2$	Tyr(OCH3)NHCH$_3$	RSS		
127	2A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OBZ)NHCH$_3$	RSS		172-175
128	2A	-	Z	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OC$_5$H$_{11}$^{n})NHCH$_3$	RSS	81-85	155-157
129	2A	ZPro	Leu	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH$_3$)NHCH$_3$	SSRSS		

TABLE 1 (Cont'd.)

No	PROCESS	A^1	A^2	Y	n	R^2	R^3	A^3	STEREO-CHEM	MP. °C $R^1=OCH_3$	MP. °C $R^1=OH$
130	2A	ZPro	Pro	NH	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH_3)$NHCH_3$	SSRSS		
131	2B	-	HO_2C	-	2	H	$CH_2CH(CH_3)_2$	Tyr(OCH_3)$NHCH_3$	RSS	133-135	110-120

Notes for TABLE 1:

1. Stereochemistry-optical centres labelled from left to right.

2. Of hydrated form where appropriate.

3. m.p. of HCl salt.

4. $R^1 = OC_2H_5$ not OCH_3

Gly = glycyl = $NHCH_2CO$

Phe = phenylalanyl = NHCHCO (with $CH_2C_6H_5$ on CH)

Val = valyl = NHCHCO (with $CH(CH_3)_2$ on CH)

Ph = phenyl = C_6H_5

Bz = $CH_2C_6H_5$

Z = $PhCH_2O.CO$

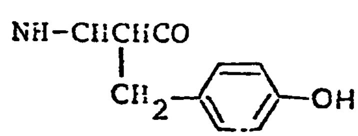
DNP = 2,4-dinitrophenyl

Leu = leucyl = NH-CHCO (with $CH_2CH(CH_3)_2$ on CH)

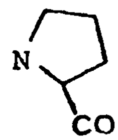
Sar = Sarcosyl = $N(CH_3)CH_2CO$

Thr = threonyl = NH-CHCO (with $CH(CH_3)OH$ on CH)

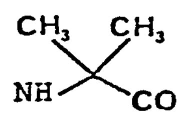
Tyr = tyrosyl =



Ser = seryl = NHCHCO (CH$_2$OH on CH)

Pro = prolyl = 

Lys = lysyl = NHCHCO ((CH$_2$)$_4$NH$_2$ on CH)

iso-Aba = iso-aminobutyryl = 

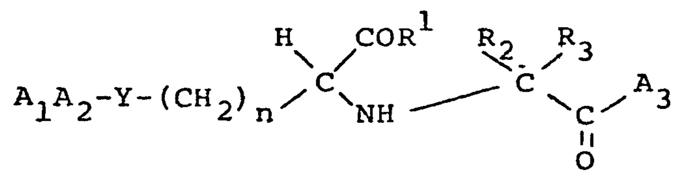
The activities of representative compounds according to the invention are given below in Table II.

TABLE II

Example No.	IC_{50} (µM) Human Rheumatoid Synovial Collagenase
5	1.7
6	42
7	5.5
9	9.5
11	0.8
13	91
14	1.2
15	3.1
16	4.9
18	1.3
19	51
21	11
22	42
23	25
24	19

What is claimed is:

1. A compound of the general formula



and pharmaceutically acceptable salts thereof wherein n is 1 to 4 inclusive;

R^1 represents hydroxy, alkoxy, aralkoxy or hydroxyamino;

R^2 represents hydrogen or alkyl;

R^3 represents hydrogen,

alkyl,

substituted alkyl wherein the substituent may be one or more of the groups selected from hydroxy, alkoxy, aryloxy, aralkoxy, mercapto, alkylthio, arylthio, alkylsulphinyl, alkylsulphonyl, carboxy, carboxamide, carboxyalkyl, carboxyaralkyl, aralkoxycarbonylamino, amino, dialkylamino, acylamino, aroylamino and trihalomethyl;

aralkyl,

substituted aralkyl wherein the substituent on the aryl moiety may be one or more groups selected from halogen, alkyl, hydroxy, alkoxy, aralkoxy, amino, aminomethyl, cyano, alkylamino, dialkylamino, carboxy, sulphonamido, alkylthio, nitro and phenyl;

or heteroaralkyl;

Y represents NR^4 wherein R^4 represents hydrogen or alkyl or Y represents a direct chemical bond;

When Y represents NR^4,

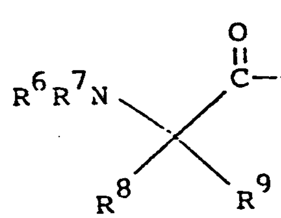
A^1 represents a group of formula R^5 wherein

R^5 may be hydrogen, alkyl, aralkyl, acyl, aroyl, aralkylacyl, alkoxycarbonyl, or aralkoxycarbonyl,

aryl,

substituted aryl wherein the substituent may be one or more groups selected from halogen, alkyl, hydroxy, alkoxy, aralkoxy, aralkoxyamino, aminomethyl, cyano, acylamino, dialkylamino, carboxy, sulphonamido, alkylthio, nitro and phenyl;

A^1 may also represent a group of the formula:



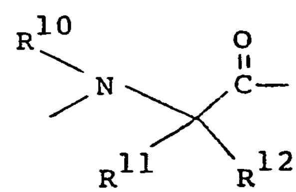
wherein R^6 represents a group having the meanings defined above for R^5;

R^7 and R^8 which may be the same or different represent hydrogen, alkyl or aralkyl; or

R^7 and R^8 may together represent an alkylene chain of 2-4 carbon atoms so to form with the adjacent nitrogen atom a nitrogen-containing ring having 4-6 atoms;

R^9 is the same as R^3 defined above.

A^2 represents a group of the formula



wherein

R^{10} and R^{11} which may be the same or different represent groups having the meanings given above for R^7 or together represent an alkylene chain of 2-4 carbon atoms so as to form with the adjacent nitrogen a nitrogen-containing ring having 4 to 6

atoms;

R^{12} represents a group having the meanings given above for R^9;

A^1 and A^2 taken together may represent hydrogen, alkyl, aralkyl, heteroaralkyl, alkylsulphonyl, arylsulphonyl, aralkysulphonyl or a group $R^{13}CO$ wherein R^{13} represents hydrogen, alkyl, aralkyl, aryl, alkoxy, aralkoxy, alkylamino. arylamino, aralkylamino, phenethenyl, phenethynyl, dialkylamino; or substituted aryl as in R^5, substituted aralkyl as in R^3 and substituted aralkoxy wherein the substituents on the aromatic moiety are as defined for substituted aralkyl

when Y represents a direct chemical bond,

A^1 and A^2 taken together represent

hydrogen, alkyl, aryl, alkoxy, aralkoxy, substituted aryl and substituted aralkoxy wherein the substituent on the aromatic moiety of the aralkoxy are as defined for substituted aralkyl,

hydroxy,

mercapto,

alkylthio,

arylthio,

aralkylthio,

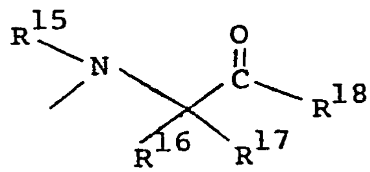
carboxy,

or carboxyalkyl;

A^3 represents a group of the formula

R^{14}

or



wherein

R^{14} represents amino,

alkylamino.

dialkylamino,

hydroxyamino,

or aralkylamino.

and R^{15}, R^{16} and R^{17} which may be the same or different represent groups having the meaning given above for R^{10}, R^{11} and R^{12} respectively and

R^{18} represents amino,

alkylamino,

dialkylamino.

substituted alkylamino wherein the substituent is amino, hydroxy, alkoxy, carboxy, carboxamido, carboxyalkyl alkylthio, alkylsulphinyl, or

alkylsulphonyl,

hydroxyamino,

alkoxyamino,

aralkylamino.

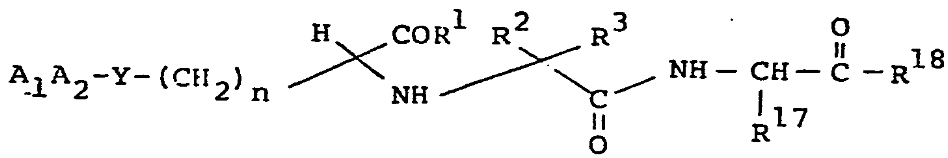
alkoxy,

aralkoxy,

or alkylaminoalkoxy,

all with the exception that when A^3 is alkylamino one of R^2 and R^3 is not hydrogen and the other alkyl or hydroxyalkyl.

2. A compound according to Claim 1 having the formula



and the pharmaceutically acceptable acid addition salts thereof wherein A^1, A^2, Y, n, R^1, R^2 and R^{18} are as in Claim 1;

R^{17} represents substituted alkyl wherein the substituent is alkoxy, aralkoxy, aralkoxycarbonylamino, carboxyalkyl, carboxyaralkyl or substituted aralkyl wherein the substituent is one or more groups selected from alkyl,

alkoxy, alkythio or aralkoxy and

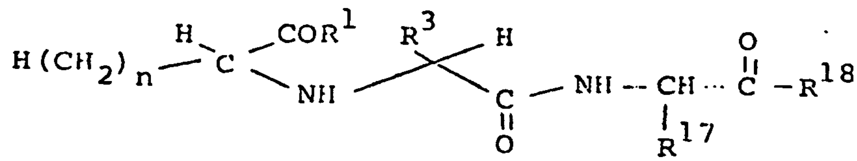
R^3 represents hydrogen,

alkyl,

substituted alkyl wherein the substituent may be one or more of the groups selected from hydroxy, alkoxy, aryloxy, aralkoxy, mercapto, alkylthio, arylthio, alkylsulphinyl, alkylsulphonyl, carboxy, carboxamido, carboxyalkyl, carboxyaralkyl, aralkoxycarbonylamino, amino, dialkylamino, acylamino, aroylamino and trihalomethyl; or

substituted aralkyl wherein the substituent on the aryl moiety may be one or more groups selected from halogen, alkyl, hydroxy, alkoxy, aralkoxy, amino, aminomethyl, cyano, alkylamino, dialkylamino, carboxy, sulphonamido, alkylthio, nitro and phenyl.

3. A compound according to Claim 1 having the formula



and the pharmaceutically acceptable salts thereof wherein

R^1 represents hydroxy, alkoxy, or aralkoxy;

n is 1 to 4 inclusive;

R^3 represents alkyl or alkyl substituted with one or two trifluoromethyl groups;

R^{17} represents substituted alkyl wherein the substituent is alkoxy, aralkoxy, aralkoxycarbonyamino, carboxyalkyl, carboxyaralkyl or substituted aralkyl wherein the substituent is one or more groups selected from alkyl, alkoxy, alkylthio or aralkoxy; and

R^{18} represents amino. alkylamino. dialkylamino. hydroxyamino, alkoxyamino, aralkylamino. alkoxy, aralkoxy, alkylaminoalkoxy or substituted alkylamino wherein the substituent is amino. hydroxy, alkoxy, carboxy, carboxamido, carboxyalkyl, alkylthio, alkylsulphinyl or alkylsulphonyl.

4. A compound according to Claim 3 wherein n, R^1,

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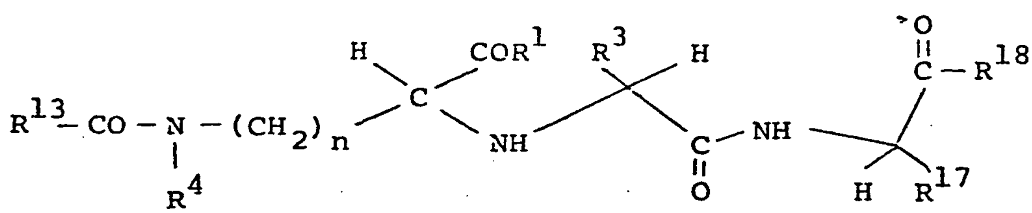
R^3, and R^{18} are as defined in Claim 3 and R^{17} represents benzyloxymethyl, 1-benzyloxyethyl, 4-benzyloxyphenylmethyl or 4-methoxyphenylmethyl.

5. A compound according to Claim 1 having the formula



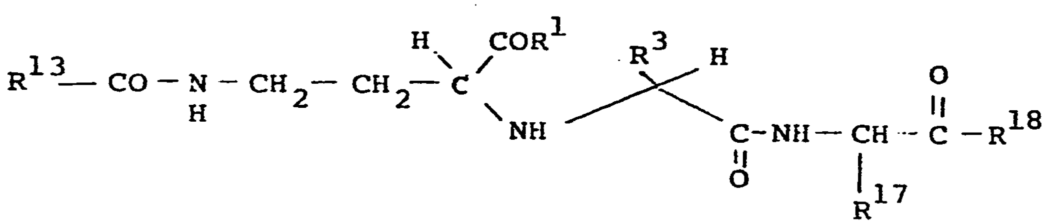
and the pharmaceutically acceptable salts thereof wherein R^4, R^1, R^{17}, R^{18}, and n are as defined in Claim 1;

R^{13} represents alkyl, aryl, aralkyl, aralkoxy, alkoxy, alkylamino. arylamino. aralkylamino. dialkylamino or substituted aryl, substituted aralkyl, and substituted aralkoxy wherein the substituent on the aromatic moiety is maybe one or more groups selected from halogen, alkyl, hydroxy, alkoxy, aralkoxy, aralkoxyamino, aminomethyl, cyano, acylamino, dialkylamino, carboxy,

sulphonamido, alkylthio, nitro and phenyl; and

R^3 represents alkyl or alkyl substituted with one or two trifluoromethyl groups.

6. A compound according to Claim 5 having the formula



and the pharmaceutically acceptable salts thereof wherein R^1, R^3, R^{17}, and R^{18} are as described in Claim 5; and R^{13} represents benzyloxy; benzyloxy substituted with 4-chloro, 2-chloro, 4-methyl, 4-nitro or 4-amino;

benzylamino;

phenyl or phenyl substituted with 4-chloro, 2-chloro, 4-methyl, 4-nitro or 4-amino.

7. A compound according to Claim 1 which is N[1-(R)-carboxyethyl]-L-leucyl-O-benzyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

8. A compound according to Claim 1 which is N[1-(R)-carboxy-3-methylthiopropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

9. A compound according to Claim 1 which is N-[4-N-(benzyloxycarbonyl)amino-1-(R)-carboxybutyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

10. A compound according to Claim 1 which is N-[3-N-(benzyloxycarbonyl)amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

11. A compound according to Claim 1 which is N-[3-N-(p-nitrobenzyloxycarbonyl)amino-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

12. A compound according to Claim 1 which is N-[3-N-(benzoyl)amino-1-(R)-carboxypropyl]-L-

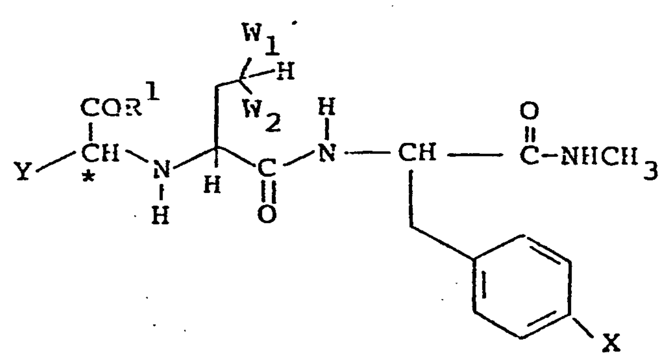
leucyl-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

13. A compound according to Claim 1 which is N-[3-(N'-benzyl)carbamoyl-1-(R)-carboxypropyl]-L-leucyl-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

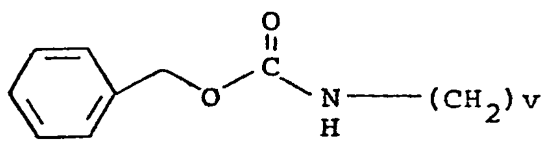
14. A compound according to Claim 1 which is N-[2-(S)-N-(1-(R)-carboxyethyl amino-4,4-di-(trifluoromethyl)butanoyl]-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

15. A compound according to Claim 1 which is N-[2-(S)-N-(3-N-(benzyloxycarbonyl)amino-1-(R)-carboxypropyl)amino-4,4-di-(trifluoromethyl)-butanoyl]-O-methyl-L-tyrosine N-methylamide and the pharmaceutically acceptable salts thereof.

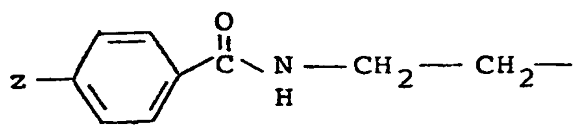
16. A compound of the formula



and the pharmaceutically acceptable acid addition salts thereof wherein x represents hydrogen, alkoxy or benzyloxy;
y represents a radical selected from alkyl, alkylthioalkyl,



wherein v is 2 or 3,



wherein z represents hydrogen or nitro; W_1 and W_2 represent methyl or trifluoromethyl; and R^1 represents hydroxy or alkoxy and the stereochemistry of the carbon marked by the asterisk is R.

European Patent Office

EUROPEAN SEARCH REPORT

0126974
Application number

EP 84104614.7

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
D,X	EP - A1 - 0 012 401 (MERCK & CO. INC.) * Claim 1 *	1	C 07 C 103/52 C 07 D 207/16 C 07 C 103/50 C 07 C 103/18 C 07 C 103/28 C 07 C 103/29// A 61 K 37/02
D,A	* Abstract *	2-16	
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P,X	EP - A1 - 0 081 094 (MERCK & CO. INC.) * Claim 1 *	1	
P,A	* Abstract *	2-16	
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D,A	EP - A1 - 0 054 862 (SCHERING CORPORATION) * Claim 1 *	1	
	--		TECHNICAL FIELDS SEARCHED (Int. Cl. 3)
A	EP - A1 - 0 050 800 (SCHERING CORPORATION) * Claim 1 *	1	C 07 C 103/00 C 07 D 207/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	27-07-1984	PETROUSEK

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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